

Understanding the ecological response of marl lakes to
enrichment: a combined limnological and palaeolimnological
approach

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I, Emma Matilda Esmeralda Wiik confirm that the work presented in this thesis is my own.
Where information has been derived from other sources, I confirm that this has been indicated
in the thesis.

Abstract

Eutrophication is a worldwide phenomenon affecting the ecology of water bodies, yet little is known of the timing, magnitude and characteristics of the resulting change in highly calcareous (marl) lakes. This project aimed to fill this knowledge gap through the combination of multi-proxy palaeolimnological analyses (last few hundred years) and comprehensive limnological monitoring (2009, 2010) of three English marl lakes, Cunswick Tarn, Hawes Water, and Malham Tarn. The specific objectives were to characterise the centennial-scale eutrophication response of macrophyte, microalgal and invertebrate communities, and to assess ecological status and reference conditions for the sites. Eutrophication-driven declines in macrophyte colonisation depth, diversity, and evenness have occurred in all sites. While such changes were expected in the most eutrophic site, Cunswick Tarn, relatively marked changes also in Malham Tarn and Hawes Water evidence high ecological sensitivity. Detailed palaeolimnological analyses of Cunswick Tarn revealed not only gradual, but also rapid and dramatic change and importantly synchronicity in response across biological groups. Shifts in community composition closely followed changes in marl deposition with (i) substantial increases over a decade in the early 1900s characterised by increases in charophytes, molluscs and plant-associated cladocerans, indicating a persistence of benthic pathways and high water transparency (ii) decreases around the 1920s, accompanied and followed by progressive increases in floating-leaved macrophytes, cyanobacteria and pelagic cladocerans. Stable isotope analysis of fossil resting eggs of *Daphnia* in Cunswick Tarn sediment appeared to indicate key changes in the balance between benthic and pelagic production through changes in both carbon uptake and patterns of *Daphnia* abundance and seasonality. Although marl lakes may initially buffer eutrophication through increased benthic production, key ecological changes identified early in the eutrophication process suggest marl lakes are as susceptible to eutrophication as other lake types. Near-complete collapse of their characteristic ecology is possible with moderate levels of enrichment.

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1 A review on the biological and chemical dynamics of marl lakes, and implications for the response of marl lakes to eutrophication

1.1 Marl lakes - general introduction

Marl lakes are a rare calcareous type of lake with unique biological and chemical properties, having formed where basins of impermeable rock are surrounded by limestone, sandstone or chalk, or in the case of for example the machair lochs in Scotland, in areas with ancient shell deposits. Marl lakes are widely distributed across the world, found in mountain areas at relatively high altitudes (Stabel, 1986; Ishaq and Kaul, 1988, 1989; Samu et al., 2010), and in lowland areas (Krienitz et al., 1996; Pentecost, 2009; Ludovisi and Gaino, 2010), which include Lake Balaton (Hungary), the largest shallow lake in Central Europe (Korponai et al., 2011). While deep marl lakes are found for example in the US (Stauffer, 1985), Switzerland (Wieland et al., 2001) and Germany (Stabel, 1986; Krienitz et al., 1996), marl lakes in the UK are mostly shallow (Pentecost, 2009).

Despite the absence of a single, overarching definition of marl lakes, there are several quantitative and qualitative descriptions based on their geology, chemistry and biology. Willby (2005) suggested conditionality of supporting carbonate-rich geology such as limestones from the Carboniferous and Silurian. Analysing data from a number of marl lakes in the UK, he developed a set of chemical characteristics for identifying such lakes (Table 1.1). This served to exclude lakes with temporary marl deposition resulting from high primary productivity. Others have suggested stricter definitions based on the carbonate (CO_3^{2-}) content of the sediment (Pentecost, 2009, and references therein), which would generally require an average of at least 50% calcite (CaCO_3). A less exclusive threshold has been suggested by Pentecost (2009) whereby marl lakes require an average surface-sediment CaCO_3 content exceeding 50 % of the dry weight of the inorganic fraction.

In terms of biological characteristics, marl lakes fall within category 3140 in the European Union Habitats scheme as "hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp." (EU-HAB 1992; EC-DG ENV 2007). A similar classification scheme has been developed specifically for lake habitats in the British Isles, with marl lakes falling within category I, "base-rich lowland lakes, with *Chara* spp., *Myriophyllum spicatum* and a diversity of *Pota-*

mogeton species” (Duigan et al., 2007; Palmer et al., 1992). Marl lakes are found across the British Isles, yet add to only <1% of the British lake total (Pentecost, 2009).

Eutrophication has been identified as the single most important threat to the ecological state of marl lakes in the UK (Pentecost, 2009). In part this is due to the sensitivity of charophytes to eutrophication, showing nationwide declines in species diversity and distribution (Stewart, 2004). The rarity of marl lakes in the UK, and their unique chemical and biological features, underline the importance of careful conservation and management, not only for achieving good ecological status as stipulated for all surface waters by the European Water Framework Directive (EU-WFD 2000), but also for preserving biodiversity and ecosystem services (Holmes, 1956; SSSI-HW 1986; McGarrigle and Champ, 1999; NI-HAP 2005). This review focuses on the chemical and biological responses of marl lakes to nutrient enrichment, drawing from studies addressing particular aspects of calcareous systems, and studies specifically on marl lakes.

Table 1.1: The typical concentration range of selected elements, adapted from Willby (2005)

Elements	Ca (mg L ⁻¹)	Mg (mg L ⁻¹)	Ca:Mg
Range	> 30 (often 50-70)	< 6	> 7 (usually 10-15)
Elements	Na (mg L ⁻¹)	K (mg L ⁻¹)	SO ₄ (mg L ⁻¹)
Range	< 10	< 3	< 20 (mostly < 10)

1.2 Chemistry as a driver of the biological communities of marl lakes

The calcareous properties of marl lakes are the result of the action of water on the surrounding limestone. Groundwater, becoming acidic after the interactions of meteoric water and soil microbial activity, dissolves carbonates that subsequently become transported into a lake. This input is continuous, with slight seasonal fluctuations depending on the amount of rainfall and soil biological activity. It affects the dissolved ion chemistry of marl lakes by introducing a high concentration of carbonate (CO₃²⁻) to the standard carbon dioxide (CO₂) dissociation reaction (5).



Carbonic acid (H₂CO₃) is formed upon the dissolution of CO₂ in water. Increasing the amount of CO₂ pushes the reaction to the right and increases the amount of hydrogen ions

(H^+), thus reducing pH. However, at equilibrium with the atmosphere, the concentration of CO_2 is stable. Dissolved $CaCO_3$ exists as ions of calcium (Ca^{2+}) and carbonate (CO_3^{2-}), of which the former is neutral in terms of pH. CO_3^{2-} , on the other hand, is a stronger base than HCO_3^- is an acid. Therefore, as CO_3^{2-} is added, it accepts and neutralises hydrogen ions (H^+), pushing the reaction to the left and increasing the pH of a solution. Carbonates act as an effective buffer against acids, which makes hardwater lakes more resistant to acidification than softwater lakes.

The high concentrations of CO_3^{2-} and Ca^{2+} also drive marl precipitation. Precipitation of marl occurs mostly in summer, when high photosynthetic activity removes CO_2 from the water column. The removal of CO_2 , accompanied by an increase in oxygen, reduces the concentration of H^+ and increases pH further to values even surpassing 9. Unlike most salts, the saturation point of $CaCO_3$ decreases with increasing temperature as well as pH. It follows that summer conditions of warm weather and high photosynthetic activity induce the precipitation of CO_3^{2-} as solid complexes (Otsuki and Wetzel, 1972). Marl may therefore consist of various CO_3^{2-} compounds, mostly $CaCO_3$ crystals but also complexes of $CaCO_3$ and organic as well as inorganic substances. Ca can also be substituted by other metals such as Mg.

The high concentration of HCO_3^- relative to CO_2 also shapes the photoautotrophic community of marl lakes. CO_2 is the preferred carbon species of most aquatic macrophytes (Allen and Spence, 1981), and species with high affinities for HCO_3^- are typically associated with eutrophic lakes, as in such conditions pH (and HCO_3^-) is high owing to particularly high levels of photosynthesis. Therefore, relatively oligotrophic marl lakes frequently contain phytoplankton associated with eutrophic lakes (Reynolds, 1998; King and Champ, 2000), which has also led to potentially misleading trophic classifications (Pybus et al., 2003). Among macrophytes, species with particularly high affinities for HCO_3^- include *Potamogeton lucens* (Staal et al., 1989), *Elodea canadensis* and *Potamogeton crispus* (Allen and Spence, 1981), and *Myriophyllum spicatum* (Hutchinson, 1970). Charophytes, macrophytic algae, are also included in this group (Kufel and Kufel, 2002; Hidding et al., 2010). Consequently, these species, especially the charophytes, are frequently the dominant macrophytes in marl lakes (Spence et al., 1984; Willby, 2005; Free et al., 2007). *Fontinalis antipyretica* (a moss), *Hippuris vulgaris* and *Potamogeton polygonifolius* are relatively poor at assimilating HCO_3^- (Allen and Spence, 1981), and this may in part explain their association with relatively unimpacted (marl) lakes (Willby,

2005).

Charophytes and marl lakes are commonly associated with clear-water, low-nutrient conditions especially in summer during peak marl formation. This is mainly due to a reduction in the abundance of phytoplankton by two mechanisms. Firstly, phosphorus (P) incorporation into marl crystals (coprecipitation) reduces bioavailable P and therefore phytoplankton abundance, and secondly, marl forming around phytoplankton induces sinking and senescence (Stabel, 1986; Koschel et al., 1987). Traditionally, marl lakes have been claimed to be capable of buffering against eutrophication due to coprecipitation (Kleiner, 1988; Robertson et al., 2007; Hamilton et al., 2009). However, in shallower marl lakes where macrophytes are a substantial proportion of the photoautotrophic biomass, buffering may not occur by similar mechanisms to deep lakes.

1.3 Charophytes as environmental engineers

Charophytes can maintain low water column nutrient concentrations through a variety of mechanisms. Firstly, the action of the complexation of CaCO_3 with P plays a crucial role. Calcite accumulates on charophyte thalli and reproductive structures during the growth period, accounting for as much as 60% of their dry weight (Hutchinson, 1975, cited in Kufel and Kufel, 2002). Siong and Asaeda (2006) have estimated that 14 - 21% of *Chara*-associated P can be immobilised within the CaCO_3 crystal structure as opposed to adsorbed on the surface. Thus, charophytes effectively remove P from the water column during a critical period of growth for most plants, reducing competition with other species including phytoplankton. Charophyte biomass is also particularly resistant to decomposition, which immobilises nutrients beyond the senescence of charophytes at the end of the growth period and maintains low nutrient concentrations (Krolikowska, 1997; Kufel and Kufel, 2002).

Secondly, most aquatic macrophytes rely predominantly on sediment for the uptake of nutrients (Barko et al., 1991). Owing to their modest rhizomes and absence of conducting tissue, transportation of nutrients between the rhizomes and growing tissue of charophytes is inefficient and requires energy. Consequently, charophytes would be expected to assimilate nutrients both from the water column and sediment. In support, Vermeer et al. (2003) found charophytes to predominantly collect nutrients from the water column in the laboratory. Kufel and Ozimek (1994) found through a series of experiments that *Chara aspera* were, at rela-

tively high densities, able to reduce water column soluble reactive phosphorus (SRP) by over 90% after 24 hours from an initial SRP of $200 \mu\text{g P L}^{-1}$, and suggested *Chara* are important in controlling P turnover in lakes. This effect may be enhanced by the flexibility of P uptake in *Chara*, as more P is assimilated with increasing P concentrations (Rees et al., 1991; Kufel and Ozimek, 1994). In the absence of P in the water, however, sediment uptake can be efficient (Wüstenberg et al., 2011). Efficient P uptake, specifically from the water column, maintains low ambient nutrient concentrations and serves to further suppress phytoplankton production in a lake. While the existence of allelopathic effects of *Chara* on certain phytoplankton has been demonstrated, their significance in natural environments is uncertain (van Donk and van de Bund, 2002).

Finally, charophytes can reduce ambient nutrient concentrations by virtue of growing as dense stands, which reduces wind and wave stress and subsequently sediment resuspension and dispersal (Kufel and Kufel, 2002). Many charophytes are perennial, also found growing under ice, and therefore their negative effect on nutrient availability for phytoplankton can continue throughout the year (Krolikowska, 1997; Kufel and Kufel, 2002; Rodrigo et al., 2007). Continuous productivity can also contribute to maintaining oxygenated sediments through O_2 delivery through rhizomes, promoting nitrogen removal (Lijklema, 1994).

It would be logical to assume that the extent to which charophytes can reduce nutrient concentrations in the water column and suppress phytoplankton depends on their abundance. Regrettably, it is difficult if not impossible to separate causation from correlation. This shortcoming notwithstanding, a number of studies have found that nutrient concentrations (Krolikowska, 1997; van den Berg et al., 1998b) and turbidity (van den Berg et al., 1998a) in the water column are reduced within charophyte stands compared with other areas, suggesting a connection between specifically charophytes and water conditions. Increases in *Chara tomentosa* cover from 10 to 40% have been associated with an overall reduction in turbidity and P in the Swedish lake Krankesjön (Hargeby et al., 1994; Blindow et al., 2002). While high water clarity in Lake Wolderwijd (Netherlands) has been found within charophyte stands in comparison with stands of *Potamogeton* spp., SRP was relatively high (Meijer and Hopper, 1997). In this case, total phosphorus (TP) and total nitrogen (TN) concentrations were smaller within charophyte stands, but not to a statistically significant extent. The expansion of charophytes (and other macrophytes) has also been suggested to have contributed to

increasing stability of water transparency in Scharmützelsee (Hilt et al., 2010).

1.4 Feedback loops involving the precipitation reaction and sediment chemistry

Marl precipitation involves various feedbacks between temperature, pH and photoautotrophs. An inherent negative feedback occurs within the precipitation reaction itself, since the removal of CO_3^{2-} reduces alkalinity as well as the reactant concentration in the water (assuming precipitation is higher than the background input of dissolved CaCO_3), thereby reducing precipitation. Interestingly, it has even been suggested that hardwater lakes be managed by nitrate addition, since this would stimulate algal production, increasing pH and thereby maintaining a low CaCO_3 saturation point (Murphy et al., 1983). Other negative feedbacks on precipitation involve sulfate (SO_4^{2-} ; for example from marine spray), magnesium (Mg) (Pytkowicz, 1965) (probably not relevant at concentrations found in marl lakes), phosphates, and organic acids, which inhibit CaCO_3 crystal growth through occupation of free sites (Reynolds, 1978; House, 1990).

The “self-cleaning” mechanism of coprecipitation becomes inhibited as an increasing proportion of crystal growth sites become blocked with increasing phosphate concentration (House, 1990), and perhaps at a critical level results in the loss of eutrophication buffering capacity. Danen-Louwerse et al. (1995) found that in the laboratory, phosphate concentrations above $300 \mu\text{g L}^{-1}$ thwarted precipitation due to a buffering of the pH, preventing a rise above 8.5. This is self-reinforcing, since inhibition of precipitation becomes stronger with decreasing pH (Lin and Singer, 2006). Highly eutrophic sites may develop positive feedback loops - the more P, the less precipitation and the more remaining P, further enhancing enrichment by external P inputs. For example, in the highly eutrophic Feldberger Haussee (Germany), CaCO_3 precipitation ceased during the height of eutrophication in the early 1980s (maximal TP exceeded $1400 \mu\text{g L}^{-1}$), but resumed in 1985 following reduced nutrient loads (Krienitz et al., 1996). Cessation of marling was attributed to inhibition by high SRP concentrations. Conversely, the 10-fold decrease in maximal TP concentrations within 20 years thereafter was attributed to the combination of coprecipitation (Kasprzak et al., 2003) and a decline in overall phytoplankton biomass (Krienitz et al., 1996).

Fulvic and humic acids, as well as polyphenols, are common compounds of both terrestrial and aquatic matter (Reynolds, 1978; Grossl and Inskeep, 1992; Hoch et al., 2000), and in-

creases in surface run-off and erosion, combined with dissolved organic matter (DOM) increases through autochthonous productivity, could inhibit precipitation in marl lakes (Hoch et al., 2000). Reynolds (1978) suggested that marl precipitation in Lake Powell in the United States was inhibited by polyphenols being washed into the lake as runoff during spring and early summer. Overall, high amounts of dissolved humic substances have been found to maintain P and iron (Fe) in solution (Shaw, 1994). However, the aforementioned organics do not seem to have a significant dissolving effect on solid CaCO_3 (Oelkers et al., 2011).

The amount of P withdrawn from the water column through coprecipitation, with or without inhibition, can be significant. In some lakes, removal concentrations of $100 \mu\text{g L}^{-1}$ SRP have been reached during algal blooms and overall high levels of photosynthesis (Murphy et al., 1983). In laboratory experiments, 74% of phosphates have been found to precipitate with CaCO_3 at pH 9.5 - 10 (Otsuki and Wetzel, 1972). Kleiner (1988) estimated coprecipitation to account for about 35% of the epilimnetic P removal in Lake Constance (Germany) (although see also Hupfer et al., 1995, regarding negligible sedimentation of coprecipitated P). Coprecipitation has been attempted as a restoration method in eutrophic lakes by the artificial application of lime. Interestingly, while there are examples of success (Ghadouani et al., 1998, and references therein), there is also evidence to the contrary, such as the failure in the naturally calcareous Arendsee (Germany) (Hupfer et al., 2000). Laboratory experiments, and evidence of short-term limnetic coprecipitation, despite offering insight into the eutrophication-buffering potential of coprecipitation, do not necessarily capture the whole cycle - the ultimate fate of coprecipitated P depends on diagenetic processes in the sediment.

1.4.1 Short-term changes in sediment-level chemistry

The amount of P that is released from the sediment is difficult to predict due to the incomplete understanding of sediment chemistry. Oxygen, pH, redox conditions and sediment metal composition all influence P release and retention, but experiments on the effect of varying each of these parameters have produced conflicting results. In addition, comparatively little is known on the details of microbial processes in the sediment layers. The main fields of contention involve oxic vs. anoxic P release, and the role of CaCO_3 and Fe for P retention in the long and short term.

While an increase in temperature enhances marl precipitation and P immobilisation, it simul-

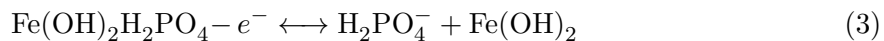
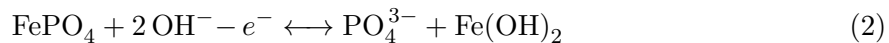
taneously enhances sediment mineralisation and P release (Jensen and Andersen, 1992), the two effects possibly canceling each other out (Boström et al., 1988). Jensen and Andersen (1992) have found that temperature can explain more than 70% of the seasonal variation in internal loading in four Danish lakes, thereby diminishing the importance of other P release processes.

Redox and pH at the sediment-water interface can in theory affect P binding in a number of ways. Redox potential is an expression of the tendency of an environment to accept or donate electrons. This tendency is partially dependent on the H^+ concentration, which links redox with pH such that high pH reduces the maximum attainable redox potential within the stability limits of water (DeLaune and Reddy, 2005). However, low and high redox potentials can exist at low and high pH. Redox importantly drives the solubility of a number of metals that complex with P. For example, Fe and aluminium (Al) can exist in various oxidation states. At high redox potential, Fe and Al can complex with oxygen to form Fe/Al oxides (including P complexes), whereas at low potential Fe oxides can dissociate, forming Fe^{2+} in solution (Golterman, 2001), with Al oxides slightly more stable (Kopáček et al., 2005). Waters are more reducing at low oxygen concentrations, and conversely, more oxidative at high concentrations. The relationship between oxygen, pH, redox, and metal solubility, forms the basis of theories on oxic and anoxic P release in sediments (see e.g. Golterman, 2001).

Fe-bound P is commonly expressed as $Fe(OOH)\sim P$, and involves Fe in the oxidised Fe^{3+} state. Traditionally, this is the form of P claimed to be released when redox potential declines. However, $Fe(OOH)\sim P$ is more thermodynamically stable than $FeOOH$, and fairly redox-insensitive, suggesting that within the redox potentials naturally occurring in lakes, it is unlikely to dissolve (Golterman, 1998). What has been interpreted as Fe-bound P may instead be microbial in origin, or microbially mediated (Jansson, 1987; Burley et al., 2001; Golterman, 2001; Hupfer and Lewandowski, 2008; Huang et al., 2008). Also, high concentrations of sulfur (S)-containing molecules can result in the formation of FeS under low redox potential, releasing P (Eckert et al., 1997; Golterman, 2001). Importantly, S cycles have also been evoked to explain the failure of hypolimnetic oxygenation to reduce internal loading of P (Gächter and Müller, 2003), thereby eroding the “oxygen rule”. In support, P release rates from sediments with changing oxygen concentrations have often been found to be fairly constant (de Montigny and Prairie, 1993; Hupfer et al., 1995; Hupfer and Lewandowski, 2008,

and references therein). There is consequently no overarching rule on the release of P from anoxic vs oxic sediments.

An increase in sediment-level pH may enhance release of P through OH^- -exchange with sedimentary metals by effects on the following reversible equations (Huang et al., 2008):



The resulting increase in P release has been found to as much as double between pH 8 and 9 (Boers, 1991). To the contrary, only one of four lakes studied by Jensen and Andersen (1992) responded to an increase in pH by an increased P release (pH range 7.5 - 10.5). At the opposite end of the spectrum, a decrease in sediment pH can cause the dissolution of Ca-bound P due to the dissolution of the Ca mineral structure. The binding properties of Fe and Ca have been researched in the laboratory by Golterman (1988) who found that the high pH associated with marl lakes promotes Ca-P over Fe-P binding, but the pH effect does not necessarily extend into the sediments. Here, adsorbed P is transferred to Fe (Figure 1.1, from Golterman (1988)), which further emphasises the importance of Fe in determining the potential of internal loading in a lake (see section 1.4.2).

Heterotrophic microbes react to changes in sediment conditions since their metabolism depends on the availability of electron acceptors for respiration. Owing to the high abundance of microbes in sediments, the fate of P may be largely dependent on microbial cycling, mediated by macro-organisms (see for example Phillips et al., 1994; Hines, 2006; Huang et al., 2008). Whilst microbial activity is also high under oxic conditions, resulting in recycling of nutrients, a shift from oxic to anoxic conditions frequently results in elevated P release (Eckert et al., 1997; Spears et al., 2007; Jiang et al., 2008). This in part owes to fermentation requiring more organic material than oxidation for a given amount of energy output, resulting in the release of excess P (Golterman, 2001). The type of activity at the sediment-water interface depends also on light. In shallow lakes with good light penetration, the sediment can be dominated by photoautotrophs, which immobilises P and stabilises the sediment. With decreasing light, the sediment becomes increasingly dominated by heterotrophs and is more likely to release P (Spears et al., 2007). This makes eutrophic lakes with low light penetration and high produc-

tivity in the plankton potentially more prone to internal loading, compounding the effects of external loading.

The long-term P retention of sediments depends on the capacity to hold P in recalcitrant forms, mainly in anoxic environments since even sediments in well aerated environments will become anoxic with depth. What are the recalcitrant forms of P in view of the factors discussed above?

1.4.2 Long-term sediment phosphorus dynamics, and iron vs calcium

The capacity of sediment to incorporate P is limited, not infinite. A few methods have been developed to assess the binding capacity of sediments. One empirical method involves measuring the sorption capacity of a sediment, defined as the maximum P retained in a sample of sediment upon loading with a range of SRP concentrations (4).

$$S_c = S_{max}k_L C / (1 + k_L C) \quad (4)$$

where, in general terms, S_c is the calculated sorbed P, S_{max} is the maximum P binding capacity, k_L is a fitted constant related to the binding energy of the sediment, and C is the concentration of P at time n .

Another method, used by palaeolimnologists, is based on the interpretation of various theories on sediment P binding with certain elemental ratios used as proxies for the binding capacity of a sediment and past redox states. Examples include Al:Fe, Al:P (Kopáček et al., 2005), Fe:P (Jensen et al., 1992), and Fe:Mn (manganese) (Hobbs et al., 2005, and references therein). However, the use of these ratios is constrained by uncertainties regarding their specificity as proxies and the mobility of elements in the uppermost sediment layers. In addition, the uncertainties concerning short-term P release mechanisms also plague the interpretations of long-term P dynamics. P coprecipitated with marl is often expressed as immobile, or redox-insensitive, and claimed to be important for the long-term storage and thus withdrawal of P from the water column (for example Pan and Brugam, 1997; Ishaq and Kaul, 1988, 1989; Robertson et al., 2007). This has been used as the argument for the eutrophication buffering capacity of marl lakes, since their sediments can consist for the most part of carbonates.

Assuming that marl lakes deposit immobile forms of P, it is interesting that a number of

studies on marl lakes have demonstrated that long-term retention of P depends more on Fe than CaCO_3 , with surface sediment CaCO_3 having very little, if any, impact on retention. Hobbs et al. (2005) used the Fe:P ratio and an experimentally derived maximum sediment sorption capacity to explain the patterns of eutrophication in Lough Carra (Ireland). Studies on Danish shallow, eutrophic lakes with varying Ca concentrations found evidence for most P storage being dependent on the concentration of sediment Fe (Jensen and Andersen, 1992; Jensen et al., 1992). Williams et al. (1971b) found that CaCO_3 did not explain any variation in sediment inorganic P that could not be explained by Fe. Other studies supporting the overriding importance of Fe include Williams et al. (1971a), Stauffer (1985) and Stauffer (1987). Theoretical predictive models of in-lake TP have been found to underestimate winter TP, and overestimate summer TP, in the water column of calcareous lakes in Wisconsin (Stauffer, 1985), supporting poor long-term binding of P with CaCO_3 despite summertime coprecipitation. Stauffer (1985) included non-calcareous lakes in the analysis, concluding that calcareous lakes indeed seemed more sensitive to eutrophication, and that this could probably be attributed to their low Fe content, exacerbated in cases with high SO_4^{2-} concentrations promoting FeS deposition. These findings have been supported by a more recent study including two of the lakes in Stauffer's analysis (Hoffman, 2009).

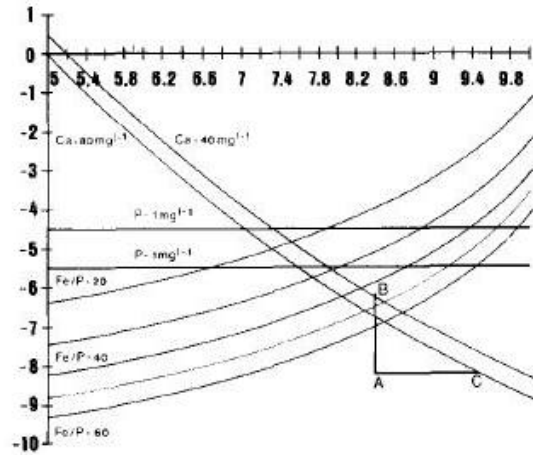


Figure 1.1: The pH-dependent binding of P

While CaCO_3 may immobilise P during the growth period through elevated coprecipitation and high pH, pH below and at the sediment surface is not necessarily as high as in the upper water column, promoting dissolution as described in section 1.4.1. The sediment may have

lower pH for long periods of time, for example in winter or during summer stagnation, resulting in CaCO_3 dissolution and P release (Gonsiorczyk et al. 1997; Burley et al. 2001; Spears et al. 2007; Jim Marshall, pers. comm. 2008). Bathymetry and stratification conditions should be taken into consideration when assessing the resistance of a marl lake to eutrophication, since they will affect the specific conditions in the sediments. Deep lakes with limited shallow surface area are more likely to have large areas of limited light penetration, low pH, and low oxygen concentrations in the profundal sediments, resulting in a dependence on Fe for long-term P storage.

Shallow lakes, on the other hand, are more likely to have more oxygenated, light-exposed sediments with relatively high pH promoting CaCO_3 deposition. However, even shallow lakes may experience CaCO_3 dissolution (Coletta, 2004). No thorough study on bathymetry-dependence of Ca vs Fe-binding has been found. There is indirect support for this, however, in a study on P dynamics in littoral and profundal sediments of a shallow, charophyte-rich high-alkalinity lake in Denmark: littoral sediments had a higher concentration of Ca-bound P than profundal, such that Fe-bound P constituted 33% more of sedimentary TP in the profundal than in the littoral sediment, while Ca-bound P was 45% higher in the littoral sediment than in the profundal sediment (Andersen and Ring, 1999). A fairly recent study concluded that the Fe:P/Ca:P ratio can be an indicator of a lake's susceptibility to acidic vs. alkaline P release (Huang et al., 2005). Mainly, Fe-P/Ca-P ratios less than 0.5 resulted in higher release of P under acidic conditions and vice versa.

Gonsiorczyk et al. (1998) compared sediment cores of two calcareous lakes in Germany, the eutrophic (Feldberger Haussee) and the oligotrophic (Lake Stechlin). While the sediment of the eutrophic lake contained higher concentrations of Ca-bound P, it contained less redox-sensitive P (Fe + Mn and organically bound P) than the oligotrophic lake sediment. The difference in Ca-associated P was in part attributed to the concentration-dependence of coprecipitation whereby coprecipitation is low at low P concentrations. The difference in the forms of P in the two sediments are coherent with theory whereby eutrophic lake sediments have a higher likelihood of experiencing low redox potential and heterotrophic P release during stratification, resulting in an increase in the release of redox-sensitive P and a relatively higher retention of CaCO_3 P. The most striking finding of the study was that TP in the sediments of the two lakes were similar. This would suggest that, regardless of the form of P in the

sediment, there is not necessarily more P in the sediments of eutrophic lakes. Calcite and other P binding forms may therefore, in some instances, function reciprocally, diminishing the buffering effect of coprecipitation in marl lakes.

Perhaps coprecipitation is predominantly a temporal and local eutrophication buffer, operating within P binding constraints. This is in agreement with the conclusions of Hupfer and Lewandowski (2008) regarding the balance between the binding capacity of sediment and external P load, whereby through excessive nutrient loads and increased autochthonous productivity, organic matter accumulates and P is released regardless of oxic or anoxic sediment conditions, while P is retained in oligotrophic lakes (Wilson et al., 2010). The P-binding capacity of marl lakes may in the long term be compromised, not by anoxia itself, but by an increase in sediment organic matter followed by increased microbial recycling of P, in turn followed by decreases in sediment-level pH and the dissolution of CaCO_3 , involving further P release. Consequently, marl lakes may resist eutrophication by increased incorporation of P into CaCO_3 up to a threshold (Gonsiorczyk et al., 1997), which may be lake-specific.

1.5 Baseline nutrient concentrations and reference states of marl lakes

Lakes in their natural state receive externally derived nutrients through atmospheric and catchment processes, which is regarded as fairly stable in time in the absence of events such as rapid changes in climate or weather, or fire. Such lakes are said to contain baseline nutrient concentrations, and exist in a reference state in terms of ecology. Identifying reference lakes and characterising comparatively impacted sites has been an important part of the European Union Water Framework Directive (WFD) (EU-WFD 2000; Bennion, 2004; Carvalho et al., 2006; Lyche Solheim et al., 2008). To this end, lakes have been typed according to depth, geographical region, and alkalinity. Generally, P concentrations have been found to increase with increasing alkalinity (Vighi and Chiaudani, 1985), leading to higher chlorophyll a (chl a) and TP reference concentrations for high-alkalinity lakes (Carvalho et al., 2006). Because coprecipitation of P with CaCO_3 serves to reduce bioavailable P and chl a concentrations in reference-state marl lakes, Willby (2005) suggested a distinction should be made between marl-precipitating and non-marl-precipitating high-alkalinity lakes. Further, Willby (2005) suggested that reference concentrations for marl lakes be similar to those set for moderate-alkalinity lakes (Carvalho et al., 2006) (Table 1.2).

Table 1.2: Various supporting standards for designating ecological status in marl lakes based on Carvalho et al. (2006) and UKTAG 2008. “Very shallow” and “shallow” indicate depths <3 m and 3 - 15 m, respectively. G/M boundary for colonisation depth is calculated as 0.75*reference depth for charophytes (Carvalho et al., 2006).

Index	Unit	Reference		Good/Moderate	
		Shallow	Very shallow	Shallow	Very shallow
chl a	μgL^{-1}	7.6	10.1	15.2	20.3
TP	μgL^{-1}	<9	<10	20	24
colonisation depth	m	9.4	4.5	7.05	3.38

The focus on P concentrations in ecological status and trophic index assessments results from the widely held notion that most temperate freshwater lakes at present are limited by P rather than N and are expected to respond more strongly to increased P loads. The parallel use of chl a as an index of trophic/ecological state is based on historical observations of strong correlations between the two. However, not only is this correlation biased by tautology, but the conclusion may not always hold, particularly for lakes in a near-natural state, and lakes in montane areas where N limitation has frequently been observed (see Lewis Jr. and Wurtsbaugh (2008) for a comprehensive review). It is also important to note that atmospheric deposition has contributed N to freshwaters for a long time and will continue to do so (Schöpp et al., 2003), reaching and fertilising remote lakes globally where direct human impact is negligible (Bergström and Jansson, 2006; Enders et al., 2008; Baron et al., 2011; Holtgrieve et al., 2011), placing the concept of modern reference state lakes under question.

1.6 Ecological responses to phosphorus and nitrogen loading, with emphasis on calcareous lakes

1.6.1 General introduction

The simple scenario of eutrophication is based on increases in productivity and biomass. This involves increases in phytoplankton, which results in reduced light penetration in the water column. This in turn induces a shift in macrophytic communities from low-growing isoetids, mosses and charophytes to taller-growing elodeids and floating-leaved species. If eutrophication progresses, declines in macrophyte coverage may lead to the complete dominance of phytoplankton, i.e. a turbid state. These changes are summarised by for example Ozimek and Kowalczewski (1984); Scheffer et al. (1993); Hargeby et al. (1994); van den Berg et al. (1999); Søndergaard et al. (2010). Distinct eutrophication-driven community composition changes

also occur among zooplankton and microalgae, allowing their use as indicators of ecological and trophic status (see for example De Eyto et al., 2003; McGowan et al., 2005; Davidson et al., 2007, 2010).

In terms of macrophyte biodiversity, eutrophication seems to trace a unimodal curve where species richness is higher under mesotrophic than oligotrophic or eutrophic conditions (Pening et al., 2008a), and species losses due to eutrophication have occurred on national levels (Sand-Jensen et al., 2000; Joye et al., 2002; Stewart, 2004). Dodson et al. (2000) found a general pattern of peak phytoplankton species richness preceding that of macrophytes along a gradient of lake productivity. Dodson et al. (2000) explained the pattern through a thermodynamic model developed by Oksanen et al. (1981), whereby the higher specific net primary productivity of phytoplankton allows higher productivity for a given input of energy compared with macrophytes with resource-demanding non-photosynthetic tissue. Interestingly, as charophytes are algae, and have high photosynthetic surface area in combination with modest rhizomes, their high richness typically under oligotrophy appears in agreement with this pattern.

Changes in species abundance and diversity may be a response to an interaction of P and N increases - for example, for a range of Danish lakes within intermediate to high P concentrations, macrophyte abundance has been found to depend on total N concentration with a threshold of 2 mg L^{-1} (González Sagrario et al., 2005). James et al. (2005) found N concentrations to show a strong inverse relationship with diversity in an analysis of 42 UK and 18 Polish lakes. Generally, there were clear differences in diversity between lakes with a winter nitrate concentration above and below $1 - 2 \text{ mg NO}_3\text{-N L}^{-1}$ (winter values are generally assumed to reflect loading more faithfully than summer values, as the latter are depleted through biological uptake and denitrification).

The sensitivity of lakes to eutrophication, and therefore individual responses to eutrophication, vary by lake morphology, involving interactions between depth, size and flushing rates. Deep lakes are relatively resistant to the effects of increased nutrient loading owing to the dilution of nutrients by a greater water volume, which is also achieved by virtue of low residence times in well flushed lakes (Vollenweider, 1975; Canfield and Bachmann, 1981; Gasith and Hoyer, 1997). Further, deep lakes undergoing seasonal stratification are less likely to exhibit phytoplankton productivity increases due to internal nutrient loading than shallow

lakes, as nutrients in the hypolimnion become largely isolated from the epilimnion (Carvalho et al., 1995). Consequently, relatively shallow, poorly flushed lakes are most likely to exhibit responses to increased nutrient loads, with internal loading being most severe in large shallow lakes where wind enhances sediment (and therefore nutrient) resuspension. However, at the other extreme, sheltered and small lakes have been found to support macrophytes at nutrient concentrations at which larger, deeper lakes have become turbid and phytoplankton-dominated (van Geest et al., 2003; Scheffer and van Nes, 2007). Small lakes tend to have smaller fetch, a relatively large percentage area of littoral habitats supporting macrophytes, and therefore habitat for pike and zooplankton, all being factors that act to reduce sediment resuspension, turbidity, and phytoplankton biomass (van Geest et al., 2003). In broad terms, medium-depth lakes, buffered neither by large areas colonisable by macrophytes nor by vast hypolimnia isolating nutrients, are most susceptible to eutrophication (Genkai-Kato and Carpenter, 2005). Moderately deep lakes with high strandline development, however, may attain relatively high % macrophyte coverage, especially where deep-growing taxa such as the Characeae are present, and therefore benefit from the influence of macrophytes on water transparency (Hilt et al., 2010).

Hysteresis, and the alternative stable state hypothesis, was introduced in 1993 and has become a widely supported theory of ecological change in shallow lakes (Scheffer et al., 1993). It emerged from the observation that turbid, phytoplankton-dominated lakes did not react linearly to decreases in nutrient concentration following management procedures. Once turbid, lakes showed resistance to ecological oligotrophication due to factors such as internal nutrient loading and positive feedback loops within the biota. In terms of nutrient concentrations, intermediate levels could therefore sustain both macrophyte-dominated and turbid systems. The shift to an alternative stable state according to the model would be fairly rapid, but a time constraint has not been explicitly defined.

There have been several studies on hysteretic responses with reference to variables other than nutrients. The extent of macrophyte cover has in some cases been hysteretically related to turbidity rather than nutrient levels (van Nes et al., 2003; Egertson et al., 2004; Ibelings et al., 2007). However, more linear relations between turbidity and macrophyte cover are also apparent (van den Berg et al., 1999). Hysteresis and linear relationships may both be indicated by some parameters, but reflect correlation rather than causality due to other un-

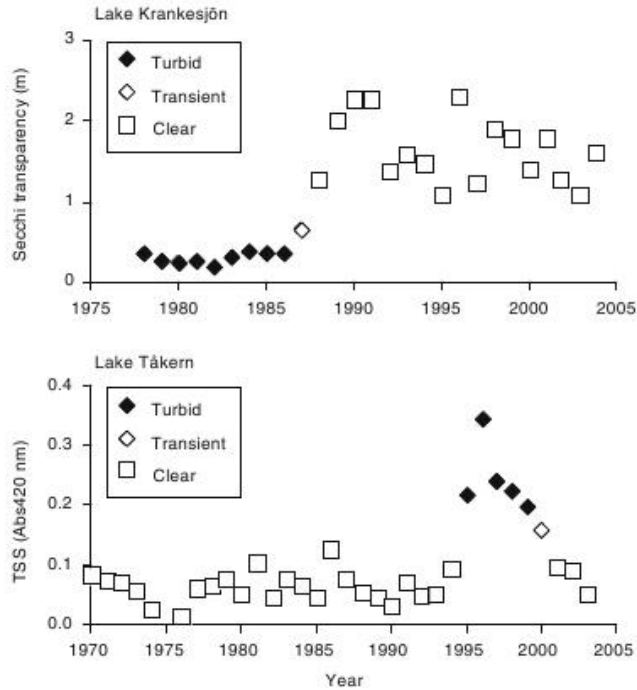


Figure 1.2: Rapid changes in water clarity in Lakes Krankesjön and Tåkern (Sweden). TSS = total suspended solids, measured spectrophotometrically (Hargeby et al., 2007)

derlying, more powerful determinands. For example, over certain ranges of TP algal biomass increases linearly, whereas algal biomass has an exponential effect on light extinction; and linear changes in chl a can have non-linear effects on macrophytes in lakes with pronounced depth gradients (Lyche Solheim et al., 2008; Phillips et al., 2008).

While rapid shifts in water clarity have been recorded in for example Swedish lakes (Fig. 1.2) (Hargeby et al., 2007), the state transition is not necessarily instantaneous, but may rather manifest itself by oscillations between states (Egertson et al., 2004). Oscillations may also be characteristic of non-impacted systems, and thus do not necessarily define state transitions or regime shifts in the strict sense (van den Berg et al., 2003; Zhao et al., 2006; Titus et al., 2004; Scheffer and van Nes, 2007). For example, biota may undergo abundance oscillations largely driven by weather and climate patterns such as the North Atlantic Oscillation (NAO) and the Gulf Stream (George Hinton, pers. comm., George and Hewitt 1998; Hargeby et al. 2006, 2004; Straile et al. 2003).

How do the ecological effects of charophytes and marl precipitation compare against the above theoretical background? Assuming charophytes enforce positive feedbacks on water clarity and low nutrient conditions (Ibelings et al., 2007; van den Berg et al., 1999; Steinman

et al., 2002), shallow marl lakes in particular would be expected to follow the alternative stable states model. The decline of charophytes reduces the strength of the feedback systems incrementally, and the increased growth of canopy-forming plants inhibits charophytes further, supporting a self-sustaining increasingly turbid system (see van Nes et al. (2003) for a eutrophication model analysis involving competition between *C. aspera* and *P. pectinatus*). Rapid shifts could also be expected in deeper marl lakes despite reduced contributions of charophytes to overall productivity, as a threshold in coprecipitation could tip the lake from the positive feedback of nutrient immobilisation to a positive feedback involving phytoplankton. Is there, then, a general breaking point of the collective function of charophytes and marl precipitation, or is the function gradually reduced in strength without the proverbial flip into an alternative state?

1.6.2 Charophyte declines: Nutrients, light and other factors

Several charophytes are included in the list of species identified to decline with increasing eutrophication as indicated by TP (Søndergaard et al., 2010). The general pattern is for charophytes to become replaced by angiosperms with eutrophication (for example Ozimek and Kowalczewski, 1984; van den Berg et al., 1999; Hargeby et al., 1994). The opposite commonly takes place during oligotrophication (Fig. 1.3).

Laboratory experiments on the sensitivity of charophytes to increasing nutrient concentrations have shown different, but generally high, P concentration tolerance for different species (Blindow, 1988; Simons et al., 1994). The sensitivity to nitrate concentration (as nitrate-N) is also variable, with increased growth observed of *C. major* at up to $\sim 5 \text{ mg L}^{-1}$, and lower values for *C. connivens* (Simons et al., 1994). Lambert and Davy (2011) found linear decline in the relative growth rate of *Chara globularis* above NO_3^- -N concentrations of 0.5 mg L^{-1} in the laboratory. A 50 % interference of daily relative growth rate was observed at 5.6 mg L^{-1} . These values seem to compare with results from growth experiments using species commonly found cohabitant with charophytes, which makes it difficult to draw any general conclusions on the direct nutrient effects on plant succession in eutrophic lakes (Ozimek et al., 1993; Boedeltje et al., 2005).

Some field data support the experiments showing charophytes tolerant to P. Søndergaard et al. (2010) analysed macrophyte data from 300 Danish lakes, some encompassing multiple

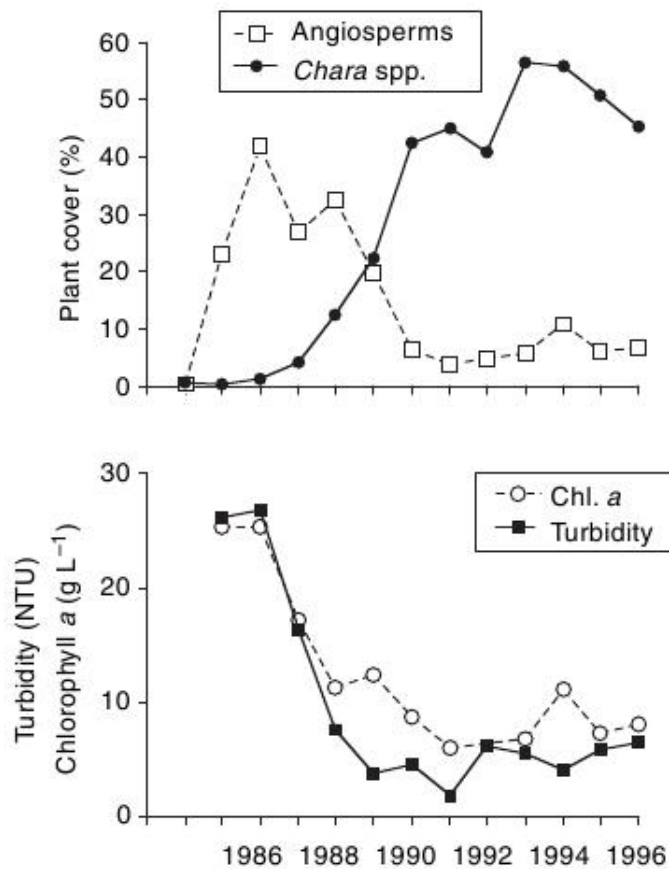


Figure 1.3: The replacement of angiosperms by charophytes during oligotrophication in Lake Krankesjön (Sweden) (Hargeby et al., 2005)

years, to establish the relationship between species composition and abundance, and nutrient variables (for example TP, chl *a*). Many *Chara* spp. were found to grow in lakes with TP concentrations up to and exceeding 200 µg L⁻¹. Charophytes with a more limited range included *Chara hispida*, *Chara globularis* var. *virgata*, *Chara rudis* and a few *Nitella* spp. The role of P in shaping macrophyte communities, especially charophytes, may be overestimated relative to N. Statistical analysis of charophyte presence, abundance and absence in 62 UK sites revealed that nitrate-N concentration, instead of SRP or various metals, explained most presence/absence patterns, with a transition from likely presence to absence occurring at concentrations of 2 mg L⁻¹ (Lambert and Davy, 2011). Charophytes in particular were disadvantaged by rising nitrate levels also in the study of Polish and UK lakes by James et al. (2005).

Charophytes have, however, been greatly reduced in abundance or even disappeared from

lakes with nutrient concentrations below the aforementioned levels (Krause and King, 1994; Davidson et al., 2002; Hargeby et al., 2007). In-lake nutrient concentrations do not therefore fully explain charophyte dynamics, but may rather have an indirect effect through effects on for example water clarity. With increasing nutrients, phytoplankton become more abundant and limit light penetration. Increased allochthonous input also reduces light penetration. Canopy-forming and/or tall macrophytes such as *Potamogeton* spp. and *Elodea* spp. can reach closer to the surface for light in contrast to several charophyte species (Blindow, 1988, 1992; Kłosowski et al., 2006; van den Berg et al., 1999). Penning et al. (2008b) analysed ecological data in a similar fashion to the aforementioned study by Søndergaard et al. (2010), but came to different conclusions, classing charophytes as sensitive to eutrophication. Importantly, the data set consisted of more than 1000 lakes from several European countries including the UK, which may indicate that the Danish results are not fully applicable on a wider scale. Since Danish lakes are mainly shallow, light is not necessarily limiting to the exclusion of charophytes, explaining the presence of charophytes at high nutrient concentrations. In support of this theory, the charophyte species with TP responses in the data set of Søndergaard et al. (2010) are associated with deeper water. Seemingly, eutrophication can be more ecologically damaging in deeper marl lakes.

The structure and chemical properties of sediment also change with eutrophication with the increase in deposition of organic matter. The looseness of organic, eutrophic sediments decreases the anchorage strength of macrophytes, resulting in a higher likelihood of uprooting and population decline (Schutten et al., 2005). Anchorage is further weakened where shorter and thicker roots are grown in response to oxygen depletion by microbial decomposition of organic matter (Raun et al., 2010). Smith (2003) confirmed similar patterns for charophytes, with decreases in shear strength linked to increasing organic matter content associated with charophyte decline. Smith (2003) also found that oospores deposited in soft sediments were likely to sink to depths of poor germination potential. Oospore germination is also prevented by H_2S , produced through microbial SO_4^{2-} reduction in anoxic, reducing conditions (Sederias and Colman, 2009).

Although charophytes are often portrayed as rapid colonisers of empty sites (e.g. Moore, 2005), their return in impacted lakes can be more gradual (Ibelings et al., 2007). van den Berg et al. (2001) found that colonisation to closed canopy densities of *C. aspera* only developed at

oospore densities exceeding 10^4 m^{-2} , suggesting charophytes cannot become abundant unless the sediment has a high propagule bank, even with satisfactory water clarity. The accumulation of oospores is a long-term process, potentially limiting recolonisation beyond existing vegetation borders. Oospore production, however, is reduced under stress through for example light limitation (Stross, 1979; Bonis and Grillas, 2002), and excess nutrients (S. Lambert, unpubl.), with low oospore numbers observed in crashing charophyte populations (J. Harris, pers. comm.), which potentially diminishes propagule banks prior to charophyte extirpation. In the Bosherston Lakes, re-establishment of charophytes in the late 1980s was not reflected in sediment oospore records, and in combination with large interannual variability in plant abundance, was interpreted as reflecting low stability and poor health (Davidson et al., 2002). However, recolonisation can be rapid where conditions are suitable (Simons et al., 1994; Blindow et al., 2002). As for mature charophytes, light conditions are also important for germling survival (de Winton et al., 2004).

1.6.3 Palaeolimnological studies on eutrophied marl lakes

Short-term studies demonstrating coprecipitation and therefore limitation of epilimnetic P (Otsuki and Wetzel, 1972; Murphy et al., 1983; Hamilton et al., 2009) may provide a snapshot only of the mechanisms operating in marl lakes, and true sensitivity may only be revealed in the long-term, especially with regards to wider, ecological responses. While long-term monitoring records of lakes are comparatively rare, information on historical change is accessible through analysis of lake sediments by means of stable isotopes, and biological remains such as macrofossils, diatoms, cladocerans, and chironomids (Smol et al., 2001). Palaeolimnology has therefore emerged as a powerful tool in eutrophication studies (Bennion, 2004; Birks and Birks, 2006; Bennion and Battarbee, 2007; Sayer et al., 2010b). Substantial centennial-scale eutrophication-related changes in biota have been recorded in a multitude of lakes (see e.g. Birks et al., 2000; McGowan et al., 2005; Rasmussen and Anderson, 2005; Bradshaw et al., 2005; Dong et al., 2012; Hobæk et al., 2012; Guilizzoni et al., 2012; McGowan et al., 2012), yet palaeolimnological publications relating to calcareous lakes, and marl lakes in particular, are scarce.

A number of deep calcareous lakes have been the subject of comprehensive research combining limnological and palaeolimnological studies, including Baldeggersee (Switzerland), Lake

Mendota (USA), Lake Ontario (Canada/USA), Nagawicka Lake (USA), and Arendsee (Germany). Deep calcareous lakes are fundamentally different from shallow lakes due to CaCO_3 precipitation focused around plankton, rather than macrophytic and/or benthic productivity. Calcite precipitation and inorganic/organic ^{13}C in particular have been studied at these sites. In general, historically recorded increases in productivity and nutrient loading correlate with increases in sediment core carbonate (CaCO_3) concentrations, as biologically induced CaCO_3 precipitation is inherently dependent on photosynthesis (Schelske and Hodell, 1991; Anderson et al., 1993; Teranes et al., 1999; Hollander and Smith, 2001; Neumann et al., 2002; Robertson et al., 2007).

While most of the aforementioned lakes have high TP concentrations (exceeding at least occasionally $100\text{ }\mu\text{g L}^{-1}$), only Baldeggersee data, through decreased core CaCO_3 accumulation rates, suggest P-inhibited CaCO_3 precipitation as eutrophication becomes more severe at TP between 300 and $500\text{ }\mu\text{g L}^{-1}$ (Teranes et al., 1999). This is in agreement with the threshold concentrations of $300\text{ }\mu\text{g L}^{-1}$ found by Danen-Louwerse et al. (1995) in the laboratory. However, even under present TP concentrations around $100\text{ }\mu\text{g L}^{-1}$, Teranes et al. (1999) suggested short-term P inhibition of CaCO_3 precipitation takes place in the spring until biological P uptake reduces concentrations to levels promoting precipitation. This phenomenon is also related to the development of strong supersaturation and particularly large CaCO_3 grains, which relates to the increasing grain size recorded in the core in correlation with increasing historical TP (Teranes et al., 1999). Increasing grain size (and therefore greater weight-to-surface area) may result in decreasing dissolution upon sedimentation (Müller et al., 2006). Interestingly, in a series of calcareous lakes in Iowa (TP range around 10 - $600\text{ }\mu\text{g L}^{-1}$), calcite precipitation as recorded in sediment cores has seemingly not decreased with time and increasing eutrophication, despite potentially inhibitory concentrations of P (Heathcote and Downing, 2012).

At Baldeggersee, Teranes and Bernasconi (2005) also investigated the balance between (anoxic) microbial, and epilimnetic phytoplanktonic productivity as indicated by the difference between ^{13}C of sediment CaCO_3 and organic matter, supporting the eutrophication model developed by Hollander and Smith (2001) based on Lake Mendota (USA) and Lake Greifen (Switzerland). The model links increased productivity and nutrient loading with water column hypoxia/anoxia, such that microbially mediated degradation of organic matter becomes more

prevalent as eutrophication progresses, overwhelming epilimnetic photoautotrophic productivity at intermediate TP concentrations until a concentration approaching $200\text{ }\mu\text{g L}^{-1}$, when the situation again reverses. Increasing anoxia entails increased production of CO_2 , which acts as an acid. Therefore, substantial development of anoxia and shallowing of oxygenated water layers may also affect the chemical function of marl lakes through effects on pH and CaCO_3 solubility (Megard, 1968).

Palaeolimnological studies of shallower calcareous/marl lakes typical of those found in the UK are comparatively few. A number of palaeolimnological studies, whilst using sediments of marl lakes, have addressed questions relating to millennial-scale climate and (terrestrial) vegetation reconstructions using pollen and isotopes (Pigott and Pigott, 1959; Pigott and Huntley, 1980; Marshall et al., 2002; Jones et al., 2002; Nuñez et al., 2002), but provide little insight into ecological change over the last 100 years or so. The few palaeoecological studies that have examined marl lake response to eutrophication over the last century indicate that successional changes in macrophyte communities have taken place along with more recent increases in pelagic productivity, suggesting that rapid eutrophication-driven biotic shifts, including those to a turbid state, have generally not occurred.

The *Chara*-angiosperm shift observed in sediment cores from several marl lakes are in support of contemporary evidence for charophyte sensitivity to eutrophication. For example, both the Bosherton Lakes in Wales (UK) (Rees et al., 1991; Davidson et al., 2002) and Ormesby Broad (UK) (Davidson et al., 2008, Figure 1.4) have undergone centennial-scale shifts from *Chara*-to angiosperm-dominated communities. Further, while reduced external nutrient loading in the Bosherton Lakes led to the return of charophytes, this was not recorded as increases in oospore numbers in the sediment record. This was attributed to poor plant health, and corresponds to other studies showing reduced oospore production under stress (Stross 1979; Martin 2001; Bonis and Grillas 2002; S. Lambert, pers. comm.; J. Harris, pers. comm.). In the very shallow Upton Little Broad (UK) (maximum depth $<1\text{ m}$) light limitation is very unlikely to develop, however *Chara* species successions and increases in pelagic cladocerans have taken place over the last two centuries (Davidson et al., 2006). In the slightly deeper Felbrigg Lake (UK), a very similar, again gradual, successional change has been recorded, including recession of macrophytes from the deeper areas (Sayer et al., 2010a). Further afield, the eutrophication history of Lake Balaton (Hungary) has been extensively researched, in-

cluding the use of subfossil cladocerans showing a record of plant-associated cladocerans in coherence with historical macrophyte abundance, revealing early eutrophication stages of increased macrophyte abundance followed by increasing productivity in the pelagic zone, again over at least two centuries (Istvánovics et al., 2008; Korponai et al., 2011).

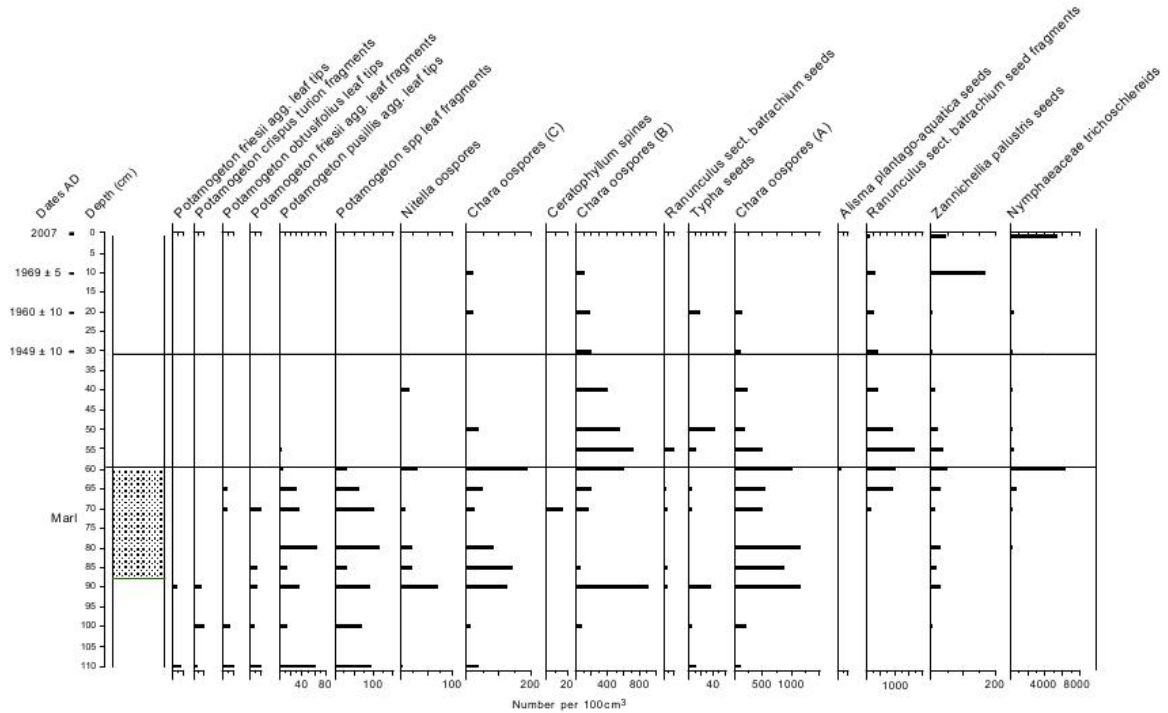


Figure 1.4: The gradual changes in the downcore macrofossil assemblage of Ormesby Broad (Davidson et al., 2008)

Several studies on marl lakes have attempted to reconstruct the recent P history of the sites based on diatom transfer functions (Anderson et al., 1993; Pan and Brugam, 1997; Garrison, 2004; Selby et al., 2005; Hübener et al., 2009). However, this method has shortcomings when applied to marl lakes, firstly due to predominantly poor valve preservation in alkaline lake sediments (Flower, 1993), which also may explain the relative paucity of palaeo-studies exploring the eutrophication history of marl lakes. Secondly there are problems associated with the application of diatom transfer functions to shallow lakes (Bennion et al., 2001), which form the majority of marl lakes in the UK. Finally, the possibility of overestimated TP concentrations owing to the high proportion of HCO_3^- in DIC and therefore relatively high presence of “eutrophic” species in marl lakes should not be disregarded (Lund, 1961; Bennion et al., 1997; Reynolds, 1998; King and Champ, 2000).

Alternative and ideally multiple proxies can be used to support palaeolimnological marl lake studies. For example, sediment pigment analysis has been shown to reflect changes in lake productivity and the composition of macro- and microphotoautotroph communities (Hertzberg and Liaaen-Jensen, 1971; Hodgson et al., 1998; Leavitt and Findlay, 1994; Vinebrooke et al., 2002; McGowan et al., 2005), with certain pigments showing relatively high specificity to particular algal groups (Hertzberg and Liaaen-Jensen, 1971; Steenbergen et al., 1994; Leavitt and Findlay, 1994). Pigment degradation is promoted by high levels of oxygen and insolation, however there is no indication to date that organic or inorganic sediments affect preservation differentially (Wetzel, 1970; Sanger, 1988; Leavitt, 1993; Mikomägi and Punning, 2007), making the method suitable for calcareous sediments.

The chitinous skeletal remains of cladocerans can be reliably identified to genus or species level (Frey, 1965; Flößner, 2000; Szeroczyńska and Sarmaja-Korjonen, 2007), and poor preservation of daphnids can in part be corrected for by the counting of ephippia, which preserve either entirely or as halves (Davidson et al., 2007). While coring location in part determines the signal given by the species in the sediment (Hofmann, 1998; Luoto et al., 2011), the remains have been shown to for the most part accurately reflect lake populations (Leavitt et al., 1994; Nykänen et al., 2009; Alric and Perga, 2011), and also give a more complete species index than water-column sampling of living organisms (Davidson et al., 2007). Subfossil cladocerans can be particularly useful in palaeoecological studies owing to their position in the middle of the food web (Eggermont and Martens, 2011), and the changes in abundance of pelagic vs benthic species associated with the loss of macrophyte beds and increases in phytoplankton (Thoms et al., 1999; Jeppesen et al., 2001).

1.7 Conclusion

The literature review suggests that marl lakes may not necessarily be more resistant to eutrophication than non-marl systems. While P immobilisation can be significant during the growing season, sediment P recycling takes place in the long term, promoting eutrophic conditions especially in deeper lakes with anoxic hypolimnia and lower pH. In shallower lakes with abundant macrophytes, increasing nutrient loads and thereby phytoplankton abundance lead to increasing competition for light under elevated nutrient concentrations, and a reduction in the standing crop of charophytes in favour of angiosperms. Such changes are further exac-

erbated by the suppression of charophyte-driven nutrient buffering. Marl precipitation may further become inhibited by high P and organic matter concentrations. High autochthonous productivity results in increasingly organic, loose sediment unfavourable to many macrophytes, and if nutrient enrichment persists, marl lakes may cease to function as marl lakes.

Despite evidence of eutrophication-driven change, most of the contemporary and palaeolimnological studies reviewed here do not suggest sudden, catastrophic eutrophication-driven shifts in marl lakes, although stepwise changes in both macrophyte and animal communities were observed in some of the palaeolimnological data. The absence of rapid shifts opens up the possibility of weak positive biotic and chemical feedbacks despite theory suggesting otherwise, although it may also be an artefact of the relatively short nutrient gradient covered by the lakes reviewed here. Owing to both a scarcity of palaeolimnological studies and the absence of a marl-lake aspect in existing studies, substantial research gaps relating to the understanding of recent anthropogenic ecological change in marl lakes remain, particularly shallow marl lakes in the UK. Further studies combining contemporary and palaeolimnological approaches are required. Increased understanding of the extent and rate of ecological change in this lake type is invaluable not only within the field of freshwater ecology, but also for conservation and management purposes.

1.8 Aims and research questions

To address knowledge gaps in the understanding of marl lake responses to nutrient enrichment, the overarching aims of this PhD project were twofold. Firstly, to elucidate the nature, time scale and magnitude of eutrophication-driven changes in biological and chemico-physical processes of marl lakes on a centennial scale, thereby establishing the sensitivity of marl lakes to enrichment. Secondly, to use the research findings to guide the assessment of marl lakes in terms of ecological status as required by the EU Water Framework Directive, including the characterisation of reference status.

Three English marl lakes, Hawes Water, Malham Tarn and Cunswick Tarn, were selected for the study, representing different morphologies and stages of eutrophication. Comprehensive palaeolimnological analyses of lake sediments over approximately the last 200 years, investigations of historical records, and contemporary monitoring of biological and physico-chemical variables through all seasons, were combined in order to answer the following specific research questions, under the following hypotheses:

- What is the current ecological status of each study site?
 - With increasing trophic status, there are reductions in macrophyte species richness, involving decreases in the seasonal duration of plant cover with associated alterations in the plankton such as increases in phytoplankton and zooplankton abundance.
- What is the reference state of marl lakes?
 - In the absence of enrichment, marl lakes exist in a stable, clear-water state, dominated by a species-rich community consisting primarily of Characeae and Potamogetonaceae.
- Has marl deposition varied over the last few hundred years, and if so, how?
 - In shallow marl lakes, encrusted charophyte remains account for a large proportion of sedimented calcite, and decreasing sediment carbonate content can be expected with increasing eutrophication following the extirpation of charophytes and increasing deposition of organic matter.

- How have macrophytic, algal, and invertebrate communities changed over the last few hundred years?
 - A transition from macrophyte to phytoplankton dominance characterises all lake types. Specifically in marl lakes, there are early declines in charophyte abundance and species richness in concert with encroachment of elodeid and floating-leaved macrophytes. Under more severe eutrophication, charophytes can disappear completely. These changes are mirrored by increased pelagic productivity, associated with increasing pelagic zooplankton and decreases in macrophyte-associated taxa.
- Has change been gradual or rapid, and does the magnitude and timing of change vary across biological groups?
 - The changes in plant and animal communities occur gradually over decadal and centennial timescales, possibly punctuated by step-like changes as observed in previous palaeoecological studies of calcareous lakes.

1.9 Structure of thesis

All data chapters contribute to the overarching aims of the study, and therefore cross-referencing takes place throughout the thesis.

The first strand of the thesis (Chapter 2) examines the contemporary and historical data from all three study sites to assess ecological status and further our understanding of marl lake ecology, characterising the former as a function of light penetration and calcite precipitation in particular. The sensitivity of marl lakes to nutrient enrichment is consequently explored, and conservation and management implications are discussed.

The second strand (Chapters 3, 4) focuses primarily on palaeoecological evidence, combined with historical records, of the centennial-scale ecological effects of nutrient enrichment in Hawes Water and Cunswick Tarn. For the most eutrophic study site, Cunswick Tarn, specific questions relating to the magnitude and rapidity of biological change are addressed through the combination of multiple palaeoecological proxies (macrofossils, x-ray fluorescence, loss-on-ignition, pigments, and sub-fossil diatoms and cladocerans) using multivariate statistical analysis.

The final strand (Chapter 5) is more methodological in nature, and examines the relatively

novel application of stable carbon isotope analysis of fossil resting eggs of Cladocera to assess eutrophication in Cunswick Tarn. Supporting stable carbon isotope data of bulk sediment, and Cladocera collected over the contemporary sampling period from all study sites, provide a broad base for comparative analysis.

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2 The limnology of three shallow English marl lakes: implications for ecological status assessments

2.1 Abstract

The assessment of ecological status of lakes is essential for satisfying the requirements of the European Water Framework Directive, with the aim to restore all surface waters to Good status by 2015. However, highly calcareous (marl) lakes in the UK have been identified as under-researched, and owing to deviations from established alkalinity-phosphorus patterns, the setting of boundary values for indices such as total phosphorus (TP) and chlorophyll *a* (chl *a*) has been uncertain. Three English marl lakes of varying morphology (shallow and very shallow) and nutrient status (meso- eutrophic) were selected for comprehensive limnological and palaeolimnological investigations with the aim to establish reference conditions and evaluate the use of WFD metrics (TP, chl *a*, macrophyte colonisation depth, and macrophyte composition indices) for assessing marl lake status. The evidence for substantial ecological change in all three sites over the previous few hundred years shows that, at least for these lakes, the performance of individual macrophyte WFD indices vary by lake type and can overestimate ecological status. Palaeolimnological and historical data are therefore valuable for assessment of status on a site-by-site basis.

2.2 Introduction

Trophic state in lakes has traditionally been defined in terms of nutrient concentrations, and nutrient boundaries have been routinely used for assessing overall water quality and degree of eutrophication (DG ENV 2010, see also Canfield Jr. et al. 1983; Lewis Jr. and Wurtsbaugh 2008 for discussions). The need to assess water quality in terms of ecological as well as chemical criteria is fundamental to the EU Water Framework Directive (WFD), which was introduced in 2000 with the aim to restore all surface waters in European Member States to Good status by 2015 (EU-WFD 2000). Good (G) is one of five categories used within the WFD to describe ecological status, the others being High (H), Moderate (M), Poor (P) and Bad (B). High status equates to reference conditions, defined as those expected in the absence of significant anthropogenic influence, while the other classes represent the degree of deviation from those reference conditions (EU-WFD 2000; DG ENV 2008). Ecological change in lakes does not display a simple relationship to nutrient boundaries, and WFD classification is there-

fore based on a set of biological elements and their supporting physico-chemical conditions conditional on lake type. As a consequence, indices for determining ecological status have been developed over the last decade (see e.g. De Eyto et al., 2003; Lyche Solheim et al., 2008; Penning et al., 2008; Jeppesen et al., 2011). These indices include chlorophyll *a* (chl *a*), total phosphorus (TP), macrophyte community composition and colonisation depth, and fish and phytoplankton communities, with lake type determined for example by alkalinity and depth (Carvalho et al., 2006).

A positive correlation between nutrient concentrations and alkalinity has been demonstrated for natural lakes (Vighi and Chiaudani, 1985), reflected by larger chl *a* and TP concentrations for a given status boundary with increasing alkalinity (Carvalho et al., 2006). However, shallow (mean depth <15 m) high-alkalinity lakes have been shown to generally exhibit suppressed chl *a* concentrations for given TP concentrations compared with other lake types (Stauffer 1985; Phillips et al. 2008, although see Robertson et al. 2007), suggesting distinct nutrient-productivity patterns. This can be attributed to the coprecipitation of P with calcite, not only limiting bioavailable P but also resulting in a coating reducing the buoyancy of phytoplankton (e.g. Stabel, 1986; House, 1990; Gonsiorczyk et al., 1998; Siong and Asaeda, 2006; Robertson et al., 2007). Where this occurs, lower chl *a* concentrations and higher TP concentrations for a given ecological status boundary could be inferred. A distinction should therefore be made between high alkalinity lakes that precipitate calcite (marl lakes), and those that do not, in order to improve the descriptive power of meta-analyses, and general understanding of lake ecology. Indeed, although marl lakes are defined in the European Union Habitats Directive (EC-DG ENV 2007), and in the corresponding scheme for the British Isles (Palmer et al., 1992; Duigan et al., 2007), Carvalho et al. (2006) identified them as under-researched and problematic, leading to uncertain nutrient/chl *a* boundaries and ambiguous lake typing for WFD purposes.

Willby et al. (2010) addressed the aforementioned research gap by including marl lakes in a category of their own in a survey of lakes in the UK, using macrophytes as indicators of ecological status, and produced similar marl lake TP boundaries as suggested for medium, rather than high-alkalinity lakes (Carvalho et al., 2006). This suggests that macrophytes may be particularly sensitive to eutrophication in marl lakes despite the consequences of coprecipitation on the chl *a* - TP relationship. Willby (2005) has further constructed macrophyte

Table 2.1: Various supporting standards for marl lakes, based on Carvalho et al. (2006) and UKTAG 2008. “Very shallow” and “shallow” indicate mean depths <3 m and 3 - 15 m, respectively. G/M boundary for colonisation depth is calculated as $0.75 \times \text{reference depth of charophytes}$ (Carvalho et al., 2006).

Index	Reference		High/Good		Good/Moderate	
	Shallow	Very shallow	Shallow	Very shallow	Shallow	Very shallow
Chl a ($\mu\text{g L}^{-1}$)	7.6	10.1	–	–	15.2	20.3
TP ($\mu\text{g L}^{-1}$)	–	–	9	10	20	24
Colonisation depth (m)	9.4	4.5	–	–	7.05	3.38

tables ranking species abundances along ecological status gradients for the UK, broadly in support of these findings. The data highlight that marl lakes in good ecological condition are typically characterised by high abundances of *Chara* and *Nitella* spp. Correspondingly, the sensitivity of marl lake macrophyte communities to eutrophication is largely driven by charophyte responses. Free et al. (2007) suggested even lower H/G and G/ M TP boundaries of 7 and $10 \mu\text{g L}^{-1}$ for Irish marl lakes, based specifically on changes in charophyte communities.

The clear water and low phytoplankton production commonly observed in marl lakes are in part attributable to the high abundance of charophytes. *Chara* spp. can become highly encrusted by calcite (Pentecost, 1984; Siong and Asaeda, 2006) and typically form dense meadows on the benthos, thereby both removing P and creating stability in the water column, and promoting benthic food webs (see e.g. the review by Kufel and Kufel, 2002). While the mechanisms behind charophyte declines during eutrophication are reasonably well understood, such as deterioration of light penetration (Ozimek and Kowalczewski, 1984; Hargeby et al., 1994; Middelboe and Markager, 1997; Schwarz and Hawes, 1997; van den Berg et al., 1999), sediment quality (Smith, 2003; Sederias and Colman, 2009) and potentially direct effects of nitrate concentrations (James et al., 2005; Lambert and Davy, 2011), holistic studies of marl lake ecology are much less common.

Comprehensive knowledge of lake characteristics is frequently attained by multivariate analyses of data sets covering large latitudinal/longitudinal and chemical/biological gradients. While these studies are valuable in understanding overarching ecological patterns, most data are snapshots of each lake, reflecting conditions of a certain season, month, and/or day only. Due to the high temporal variability of biological and chemical processes in lakes, longer-term data sets, both inter-annual (Gafny and Gasith, 1999; Hinton, 2005; Jackson and Füreder,

2006; Magurran et al., 2010) and subannual (Coveney et al., 1977; Lampert et al., 1986; Sommer, 1986; Vanni and Temte, 1990; Kahlert et al., 2002; Sayer et al., 2010), add valuable perspective to multivariate studies. For example, macrophyte cover can be highly variable within a season (see e.g. Lehmann et al., 1994) and early dieback is characteristic of lakes with increasing phytoplankton production (Sayer et al., 2010). To better control for temporal variability, site-specific seasonal and inter-annual studies are important in order to validate ecological status indices developed for the WFD, particularly as results from such classifications are used for lake management. Because marl lakes are an under-researched lake type in the UK (Carvalho et al., 2006), and they are under imminent threat from eutrophication (Pentecost, 2009), detailed information on the ecological characteristics of marl lakes in relation to eutrophication pressure is much needed.

To address these issues, this study assesses the ecological status of three English marl lakes based on a suite of WFD chemical and biological classification tools, namely TP, chl *a*, macrophyte colonisation depth, and macrophyte composition indices, using information from a comprehensive two-year limnological monitoring programme. The lakes (Cunswick Tarn, Hawes Water and Malham Tarn) are of contrasting nutrient status and basin morphometry. Ecological status as assessed by the contemporary surveys, with support from palaeolimnological analyses (two of the lakes) and historical records (all three lakes), is compared with the classification based on WFD tools. It was hypothesised that, owing to the sensitivity of charophytes to enrichment, nutrient concentrations would not provide as informative a description of the ecological status of the lakes as indices of macrophyte colonisation depth and community composition.

2.3 Methods

2.3.1 Study sites

Cunswick Tarn (CT)

Cunswick Tarn is a lowland (0.8 ha, 138 m a.s.l.), shallow ($Z_{\max} = 6.4$ m, $Z_{\text{mean}} = 3.7$ m) eutrophic (mean TP = $55.8 \mu\text{g L}^{-1}$) kettlehole lake in Cumbria, UK, and a Site of Special Scientific Interest (SSSI) (Figures 2.1a, 2.1b and 2.2a). The Tarn has steeply shelving margins with areas of shallow water restricted to the north and south ends. Most water is received from a stream at the southeast end, with minor contributions from small streams flowing off

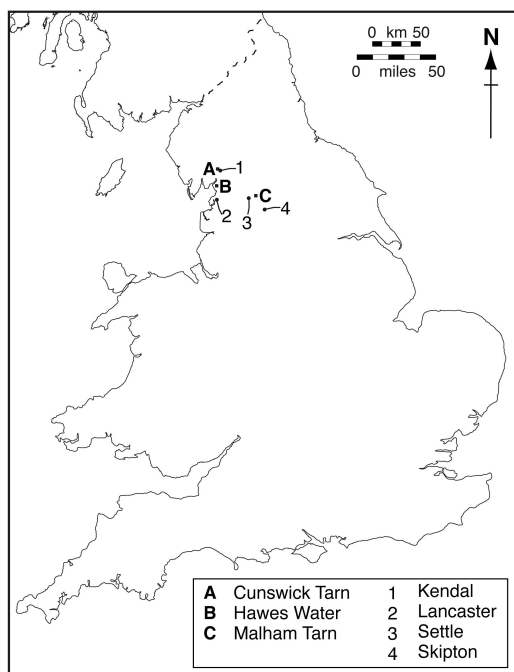
the wooded slope to the east (Figure 2.1b). All streams are high in carbonates and precipitate marl, reflected by the high in-lake mean pH of 8.17 (2-yr average). There is phosphorus- and organic-rich seepage from the woods and pastures to the north. A low-gradient, negligible outflow lies at the southwest end (Fig. 2.1b). The catchment is small and comprises mainly improved pasture, with woodland to the north and east. The Tarn is sheltered and waves form only in strong winds. While the catchment has remained largely unchanged since at least 1850, the wooded slope has occasionally been deforested. The bay at the north end has expanded over the last few years due to erosion in part owing to waterfowl, leading to locally highly turbid water and loose sediment. Further, the Tarn receives piped drainage from the improved pasture to the north, and grain is deposited at the lake margin as well as into the water to sustain an artificially large waterfowl population for recreational shooting. All these factors have resulted in nutrient enrichment of the Tarn.

Hawes Water (HW)

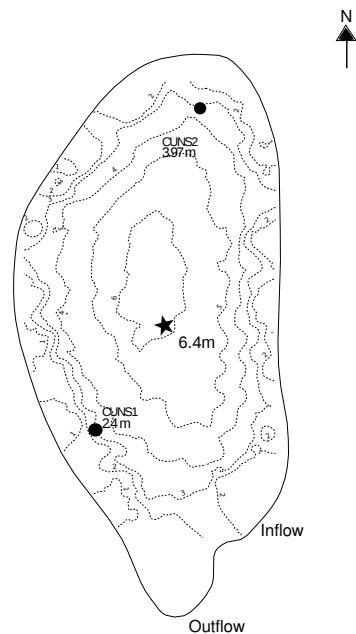
Hawes Water is a small (5.7 ha), shallow ($Z_{\max} = 12.2$ m, $Z_{\text{mean}} = 4.2$ m), lowland (8 m a.s.l.) mesotrophic (mean TP = $19.7 \mu\text{g L}^{-1}$) kettlehole lake in Silverdale, Lancashire (Figures 2.1a, 2.1c and 2.2b). It lies in a shallow basin of Carboniferous limestone in the Gait Barrows Nature Reserve and is a SSSI. The catchment is 1.7 km^2 and consists primarily of grassland and woodland, with a small number of cottages and holiday lets. The lake receives water through an artificial inflow connected to Little Hawes Water to the north, and from spring seepage from the surroundings as well as the lake bed. Annual P loads are estimated at 51.27 kg, and the retention time is approximately 0.32 years (Goldsmith et al., 2003). There is an artificial outflow with associated land drains at the southern end of the lake leading to Hawes Water Moss, the creation of which in the 19th century resulted in lowered water levels. Consequently, given the steeply shelving bathymetric profile of the lake, there is only a limited surface area of shallow water (Fig. 2.1c).

Malham Tarn (MT)

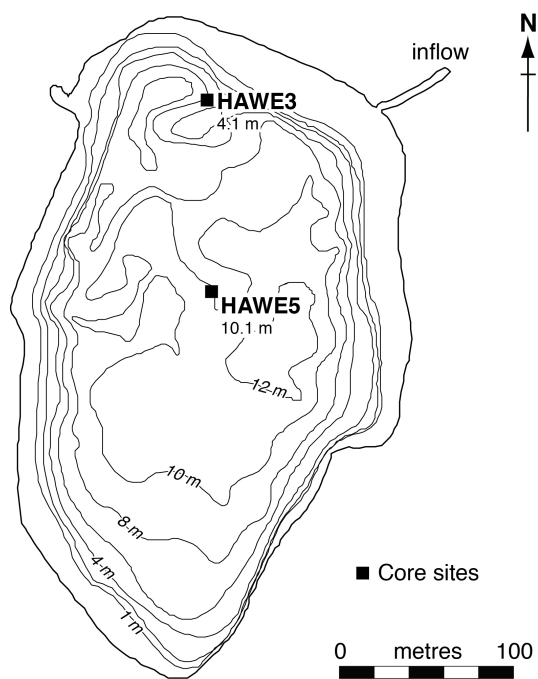
Malham Tarn is a very shallow ($Z_{\max} = 4.4$ m, $Z_{\text{mean}} = 2.4$ m), large (60 ha) mesotrophic (mean TP $17.5 \mu\text{g L}^{-1}$) lake in Craven, North Yorkshire (Figure 2.1a, 2.1d, 2.2c). At 375 m a.s.l., it is the highest marl lake in the UK and forms part of a National Nature Reserve. The Tarn is designated a SSSI, a RAMSAR site as well as a Special Area of Conservation. The 11.5 km^2



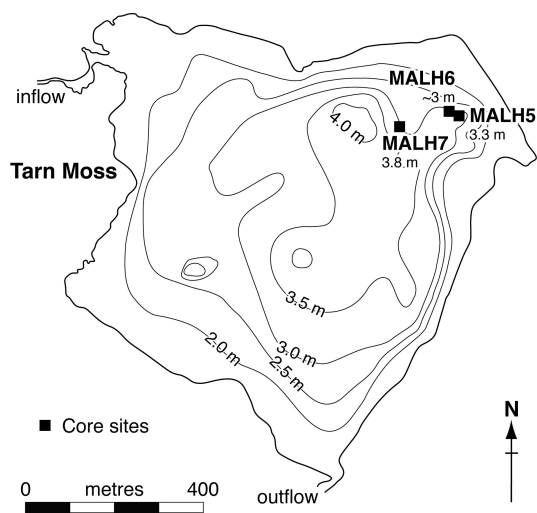
a The locations of the study sites on a map of Great Britain.



b Cunswick Tarn



c Hawes Water



d Malham Tarn

Figure 2.1: Location and bathymetric profiles of the three study sites.

catchment consists of a mixture of improved and natural cattle and sheep pastures, scattered farms, and the Field Studies Council centre lying on the north flank.

The lake is exposed, with limited sheltering to the north by a wooded slope. Dominant and often strong southwesterly winds result in a well mixed water column throughout the year. The main inflow is a large stream at the northwestern end, but the lake also receives water from springs to the north. The outflow to the south is controlled by a weir. The estimated retention time is 0.33 years, but flushing rate varies seasonally.



a Cunswick Tarn



b Hawes Water



c Malham Tarn

Figure 2.2: Study sites: CT in August 2011 from the north bank; HW in August 2009 from the southeast pier; MT from the northeast bank in June 2009.

2.3.2 Limnological monitoring

The contemporary monitoring programme was carried out for two consecutive years between February 2009 and September 2010, with one winter trip in January 2011. The following parameters were monitored monthly in spring, summer and autumn, and bi-monthly in winter: Soluble reactive phosphorus (SRP), TP, chl *a*, nitrate-nitrogen (NO_3^- -N), secchi depth, light penetration, alkalinity, pH, oxygen, conductivity, and temperature. All chemical and

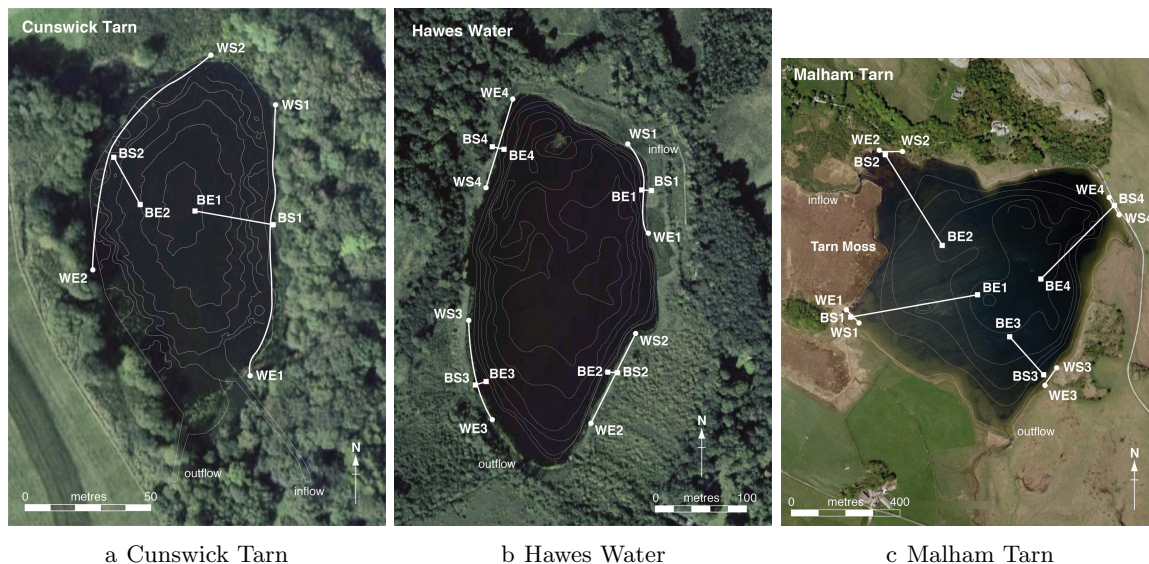


Figure 2.3: Macrophyte survey transects at the study sites. WS, WE: wader section start and end, respectively; BS, BE: boat section start and end, respectively.

physical monitoring and water collection took place at, or in close proximity to, the deepest point. Surface water was collected in acid-washed 250 ml polyethylene bottles. When weather conditions at Malham Tarn were adverse, water was collected either by a throw-bottle or by wading into waist-deep water. Chl *a* samples were collected by filtering a recorded volume of surface water through a 1.2 μm pore size GF/C filter, subsequently stored in aluminium foil and sealed in a plastic bag to prevent photodegradation and contamination. Water passed through the filter was collected for SRP and NO_3^- -N analyses. Samples were cooled to below 4°C after collection in a portable refrigerator for a maximum of four days. The samples were frozen in the laboratory and analysed as soon as possible.

Conductivity, oxygen concentration, temperature and pH were measured on-site with HACH® portable IntelliCAL probes on 10 m cables at 50 cm intervals throughout the water column. Since the probes showed different temperatures for a given depth, temperature was consistently recorded from the pH probe. Light (400-700 nm) was measured with a LI-COR® Bio-sciences LI-250A light meter on a 9 m cable, and also recorded at 50 cm intervals throughout the water column. If ambient light conditions were unstable, fewer measurements were taken. Alkalinity was measured off-site either immediately after collection or within 24 h. Titration was performed with a Hach® AL-DT test kit to the end-point of pH 4.5 using bromocresol green-methyl red as an indicator.

Cladocerans were sampled from April to September in 2009 and 2010. Due to adverse weather, May and August 2009 data from Malham Tarn are missing. Open water samples consist of five vertical trawls along a transect, performed by lowering a phytoplankton net (mesh size 100 μm) to the maximum attainable depth at each point. Littoral samples consist of three tube samples (approximately 80 cm length, internal diameter 71 cm) collected from three representative locations around the edges of each lake. Upon collection, samples were preserved in methylated ethanol until subsampling in 2011 and 2012.

In June and August of both years, semi-quantitative macrophyte surveys were conducted. The macrophyte community was monitored using a method based on standard shore and boat surveys (JNCC, 2005). At water depths ranging from 25 to >75 cm, 80 m of shoreline was surveyed with the aid of a bathyscope. Plants were identified and abundance scores between 1 and 3 were allocated. An overall abundance score for the entire subsection was given, and the type of substrate was also noted. Boat surveys departed from the centre of the shore subsection in the direction of the centre of the lake. When possible and necessary, a minimum of 10 points were scored along a depth gradient, with the aim of reaching either the maximum depth, or the maximum depth of macrophyte colonisation. The same parameters were scored as for the shore subsections. At each point, plants were surveyed and identified with the assistance of a bathyscope and species abundances were recorded. Where turbidity prevented use of the bathyscope, a double-headed rake was thrown in and trawled. The maximum depth of colonisation was determined by several rake throws. Four survey subsections were selected in Hawes Water and Malham Tarn, and two in Cunsick Tarn due to its small size (Figure 2.3a). Additional littoral and deep areas were thoroughly surveyed in Hawes Water in 2010 in an attempt to find rare charophyte species. Macrophytes were identified to genus or species level in the field. However, as charophytes are difficult to identify without microscopy, they were sent for identification to Nick Stewart (national recorder for British charophytes).

2.3.3 Water analyses

SRP, NO_3^- -N, TP and chl *a* were determined as soon as possible after collection. Frozen samples were thawed in warm water immediately preceding analysis. Spectrophotometric analysis was performed on a HACH® CAMLAB DR/4000U spectrophotometer with a 1 cm light path. Calibration and blank samples were included in all individual batches for nutrient

analysis.

SRP Soluble reactive phosphorus was analysed following the principles of the ascorbic acid method described in Glesceri et al. (1989), with a limit of detection of approximately $1 \mu\text{gL}^{-1}$.

TP Total phosphorus was determined by digesting unfiltered water in a CEM Mars Xpress microwave by the persulphate method described in Johnes and Heathwaite (1992), after which the samples were analysed following the SRP protocol. Care was taken with the addition of phenolphthalein indicator after digestion, since the dye at strong concentrations interfered positively with the spectrophotometric measurements.

NO_3^- -N Nitrate was analysed by conversion to nitrite (assuming naturally occurring nitrite to be negligible) following the method used at the UCL laboratories, according to the principles of the cadmium reduction method described in Glesceri et al. (1989), with a limit of detection of approximately 0.01 mg L^{-1} .

chl *a* Chlorophyll *a* was analysed using the spectrophotometric method described in Glesceri et al. (1989). At low light, the filter papers were ground in approximately 10 mL acetone by pestle and mortar, followed by centrifugation with a Fisher accuSpin™ 1. The method was simplified by disregarding the acidification step that separates pheophytin *a* from chl *a*, as the purpose of the analysis was to get an overall quantification of photosynthetic activity in the surface water.

2.3.4 Zooplankton analysis

Rotifers (genus/species level), cladocerans (genus/species level) and copepods (genus level) were counted from all zooplankton samples. Since cladocerans were prioritised in this study, counting was continued until approximately 100 individuals of the most common cladoceran category were identified. Counting of other groups was stopped before this point if they were very abundant and, if sparse, counting was stopped before reaching the target. For abundant samples, all counting was undertaken using a Leitz Laborlux compound microscope at 40-100x magnification with a Sedgwick-Rafter counting cell. For sparse samples, counting was undertaken using a Wild M3Z dissecting microscope with a 5 mL Bogorov counting chamber. Because of the small size of rotifers, they were not counted with the latter method. Instead,

Table 2.2: Palaeolimnological analyses undertaken for Hawes Water and Cunswick Tarn.

Lake	Methods
Cunswick Tarn	Loss-on-ignition (LOI), x-ray fluorescence (XRF), diatoms, subfossil cladocerans, macrofossils, pigments
Hawes Water	LOI, macrofossils, pigments

the first few ml were counted under a light microscope and rotifer abundance was approximated accordingly. Reproducibility of the method was tested by repeating sample counts. A fluctuation of less than 15% was standard. The raw data were converted to a concentration of $n(\text{individuals})\text{L}^{-1}$.

Zooplankton were identified with reference to Amoros (1984) and Pontin (1978). *Daphnia hyalina* agg. was used to denote all species within the *Daphnia hyalina* aggregate group due to difficulties and uncertainties of identification, and *Ceriodaphnia* were identified to genus level only (Davidson et al., 2005). Males were counted separately because they are only occasionally produced within the life cycle of cladocerans, and are associated with degenerating environmental conditions (low temperature, food limitation) (Banta and Brown, 1929; Brown and Banta, 1932). Copepods were separated into calanoids (*Diaptomus* sp.) and cyclopoids (*Cyclops* sp.).

2.3.5 Historical data

Historical data were collated from past surveys, publications and monitoring data through comprehensive web searches and personal communication. Further, palaeolimnological data for Cunswick Tarn (Chapter 4) and Hawes Water (Chapter 3) were used to support these data (Table 2.2). These data are hereafter collectively termed “historical data”.

2.3.6 Application of WFD tools

Under the WFD, the ecological status of a lake is given by the lowest score of all assessed biological parameters (fish, macrophytes, phytoplankton) following the one-out-all-out principle. Importantly, chemical parameters such as TP are used as supporting standards only. For each of the biological parameters, score designation follows a rule-based combination of the values of several subsidiary indices. In the UK, indices are calculated on a lake type basis, determined by alkalinity, surrounding geology, and average depth, classing marl lakes as a separate

lake type (UKTAG 2012). For each index, in compliance with WFD intercalibration across EU countries, Ecological Quality Ratios (EQRs) are calculated against expected reference status values for a given lake (calculations based on hypothetical expected values), the final values of which are constrained between 0 and 1. Here, macrophyte indices and chl *a* were used, the former consisting of the Lake Macrophyte Nutrient Index (LMNI), number of taxa (N_TAXA), number of functional groups (N_FG), mean proportional cover per species (COV) and proportion of filamentous algae (ALG). All indices have the same EQR value-status bins (Table 2.3) (UKTAG 2012).

TP and macrophyte colonisation depth-indicated statuses were compared with the EQR-based statuses. TP is a supporting standard and therefore does not in its own right designate status. Colonisation depth has not yet become an established method to indicate status under the WFD, and therefore beta values as suggested by Willby (2005) were used (Table 2.1). Further, WFD statuses were compared with those indicated by the Common Standards Monitoring (CSM) programme, used by Natural England, the UK government body responsible for managing SSSIs. Under the CSM, lakes are broadly classed as “favourable” or “unfavourable” with sub-classifications such as “no change” and “recovering” (JNCC, 2005). Multiple attributes fall under this scheme, including for example hydrological modifications and shoreline substrate characteristics, however for purposes of comparison with WFD status, only macrophyte- and nutrient-based attributes were used. Macrophyte assessment includes the level of infestation of invasive species, which for *Elodea canadensis* entails limit occurrences of 40 and 50% in unproductive and productive sites, respectively (updated recommendations; S. Clarke, pers. comm.). The key differences between WFD and SCM methodology are firstly, the use of TP under CSM, and secondly lake depth classes, where for SCM all lakes with a mean depth exceeding 3 m are classed as deep. Within this scheme, both shallow and deep marl lakes have boundary TPs of 20 $\mu\text{g L}^{-1}$ (updated recommendations; S. Clarke, pers. comm.). The condition assessment for SCM is also descriptive and to an extent subjective, compared with WFD assessment. It integrates palaeolimnological data where applicable, for example in determining loss of macrophyte zonation, colonisation depth, and relative abundance. In this sense, CSM is more flexible and amenable to personal observations. A site may be classified as unfavourable should any of all attributes surveyed so indicate.

Finally, hydrophyte species composition was also qualitatively compared with marl lake species

composition expected across a quality gradient (see Table 2.11, and Willby (2005) for the full list of species, and Willby et al. (2010) for more information on the tables). These tables, by indicating % abundance of a species (out of habitable, rather than total, lake area) are more sensitive to compositional change related to abundance than the aforementioned macrophyte composition metrics.

Malham Tarn is, according to WFD lake typology, a very shallow lake (mean depth <3 m), and Hawes Water and Cunswick Tarn are shallow lakes (mean depth 3 - 15 m). TP and chl *a* are the annual means of survey data from 2009 and 2010, and colonisation depth is the approximate mean of maximum depths of colonisation recorded for each sampling section during 2009 and 2010. Combined macrophyte survey data from 2009 and 2010 were used for generating the macrophyte indices, such that for example total species richness equated to the total of species recorded over the two years even if presence was not noted in both years. Further, macrophyte indices were also calculated for the lakes in the past by combining species assemblages indicated by dated historical surveys (all three sites), and plant macrofossils identified from corresponding sections of ^{210}Pb -dated sediment cores (CT, HW). This provided a basis against which to compare ecological status as indicated by current macrophyte communities. A time frame of 1900 - 1930s was chosen to represent the past of Hawes Water and Cunswick Tarn, because these years encompass several macrophyte surveys and can be matched to core sections. While no fossil record was generated for Malham Tarn, there are abundant historical records from the first half of the 1900s, and metrics were calculated for species data both from the 1920/30s and the 1950s. Where there was evidence for species presence before and after the respective time periods, the species was assumed present for this time period also, for example in the case of Potamogetonaceae which are frequently underrepresented in the macrofossil record (Zhao et al., 2006). Only LMNI EQRs, of the WFD macrophyte indices, were calculated for historical data because a full species list cannot be assumed. Therefore, the “final” WFD classifications for the sites historically are based on the EQR_{LMNI} only.

The statuses indicated by the selected WFD indices were compared with observational and historical data. Observational data include observations on filamentous algal growth, sediment texture, overall plant health, and the seasonality of macrophyte communities (Sayer et al., 2010). Observations regarding % macrophyte coverage were made in relation to available substrate (Whiteside and Lindegaard, 1982; Duarte and Kalff, 1986; Smith, 2003; Schutten et al.,

Table 2.3: EQR ecological status class boundaries for macrophyte- and chl *a*-based indices as per the WFD (UKTAG 2012)

EQR	Status
.80 – 1.00	High
.60 – .79	Good
.40 – .59	Moderate
.20 – .39	Poor
.00 – .19	Bad

2005), historical data where available, and colonisation depths for lakes of similar macrophyte composition (Spence, 1967; Jupp et al., 1974; Spence, 1982; Spence et al., 1984; Preston, 1995; Moore, 2005; Pentecost, 2009). While comparison of WFD classifications with status as qualitatively indicated by other data is inevitably subjective, the wealth of data in this study provides a firm base for qualitative assessment.

2.3.7 Statistical data analysis

The relationships between selected seasonal data were tested statistically. Owing to the brevity of the data sets, time series analysis was not performed. Instead, correlations were tested using Spearman rank correlations (chl *a* vs secchi, chl *a* vs cladoceran abundance) as well as linear regression to investigate interactions between variables likely to covary. For example, the effect of both cladoceran abundance and chl *a* was tested as a predictor of secchi depth.

2.4 Results

2.4.1 Seasonal patterns of physical, chemical and biological parameters

Carbonate precipitation on plant surfaces is most conspicuous in Hawes Water, but was also occasionally observed in Malham Tarn. Although some form of carbonate precipitation continues at Cunswick Tarn as recorded by sediment calcite concentrations exceeding 50 %, no obvious marling was observed despite pulses of high productivity in summer months and intensive carbonate precipitation in its inflowing streams. These observations are supported by patterns of alkalinity and pH data whereby carbonate precipitation is indicated by inverse trajectories - no inverse relationship is evident in Cunswick Tarn (Figure 2.4) in contrast to Hawes Water (Figure 2.5), and the pattern is weak in Malham Tarn where alkalinity was very stable through the sampling period (Figure 2.6). Alkalinity in Malham Tarn is generally

lower, and pH higher, than at the other two sites (Figure 2.7).

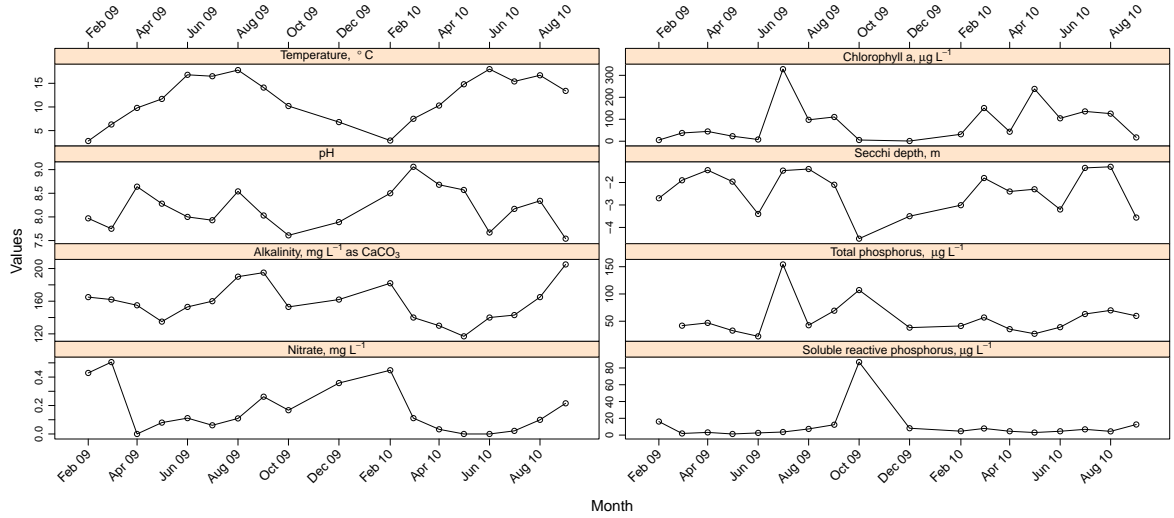


Figure 2.4: Seasonal surface water and secchi depth data of Cunswick Tarn through 2009 and 2010.

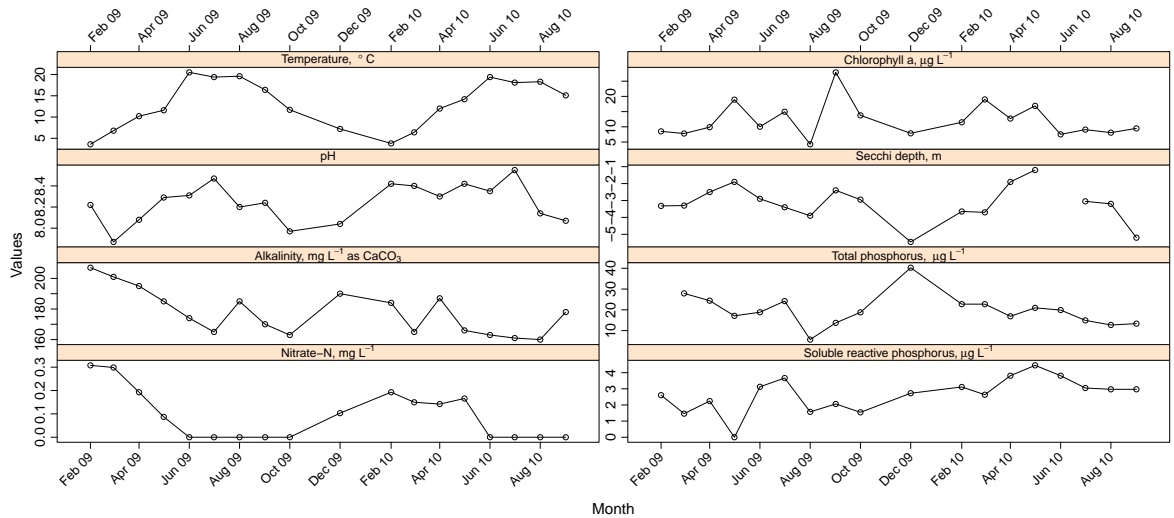


Figure 2.5: Seasonal surface water and secchi depth data of Hawes Water through 2009 and 2010.

Nutrient (TP, NO_3^- -N) and chl *a* concentrations show that Cunswick Tarn (mean TP $56 \mu\text{g L}^{-1}$, NO_3^- -N 0.19 mg L^{-1} , chl *a* $84 \mu\text{g L}^{-1}$) is much more productive than the other two sites, with Hawes Water (mean TP $20 \mu\text{g L}^{-1}$, NO_3^- -N 0.09 mg L^{-1} , chl *a* $11.8 \mu\text{g L}^{-1}$) and Malham Tarn (mean TP $18 \mu\text{g L}^{-1}$, NO_3^- -N 0.06 mg L^{-1} , chl *a* $6.4 \mu\text{g L}^{-1}$) being relatively similar (Figure 2.7). TP and chl *a* generally follow a similar pattern in summer in Cunswick Tarn and Malham Tarn (Figures 2.4, 2.6), suggesting that nutrients are largely locked up in plankton biomass. The patterns of TP and chl *a* are less coherent in Hawes Water (Figure 2.5). Ordering the lakes by mean secchi depth places the lakes in order of productivity as indicated

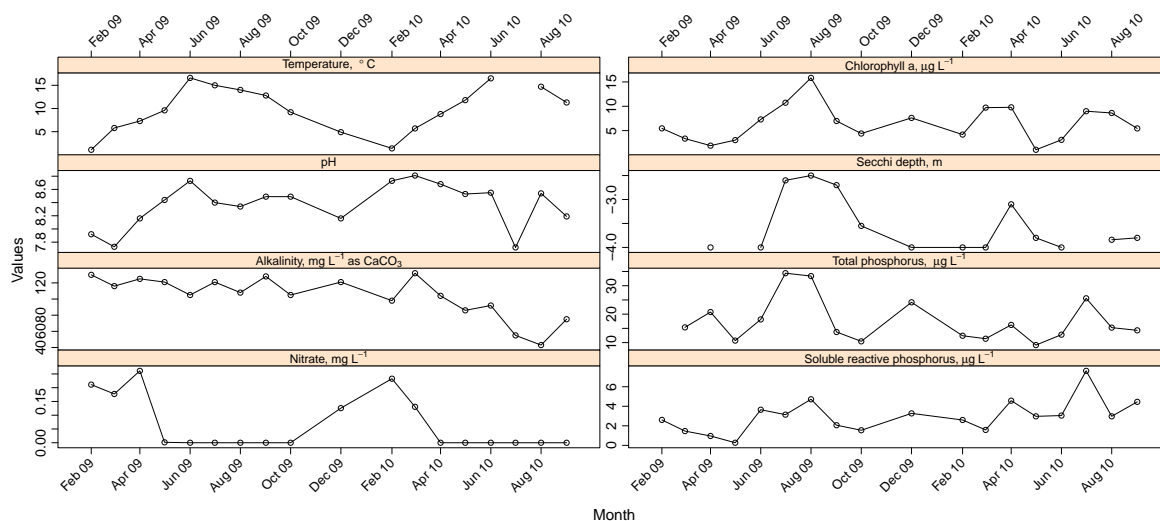


Figure 2.6: Seasonal surface water and secchi depth data of Malham Tarn through 2009 and 2010.

by TP and chl *a*. Thus, mean secchi depth is highest in Malham Tarn (3.6 m), followed by Hawes Water (3.2 m) and Cunswick Tarn (2.4 m). In all lakes, turbidity is mainly induced by

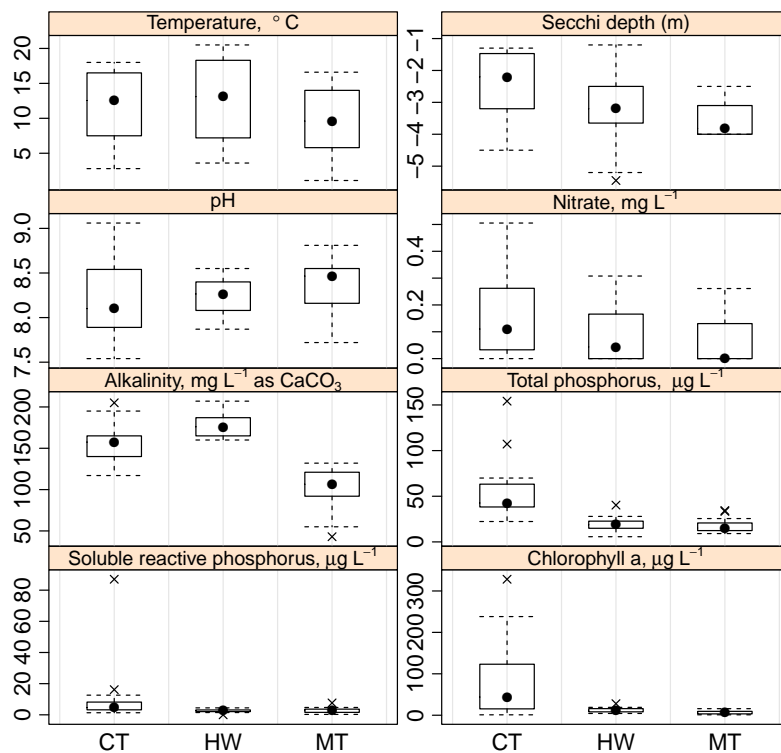


Figure 2.7: Standard box and whisker plots of water chemistry data for 2009 and 2010. Black dots are medians, box limits bound the lower and upper quartiles, and whiskers extend to 1.5 * inter-quartile range. This leaves crosses as “outliers”.

phytoplankton as indicated by low secchi depths coincident with high chl *a* values (Figures 2.4, 2.5, 2.6) and the negative relationship between secchi depth and chl *a* (Figure 2.8). This was significant for Hawes Water ($p = 0.017$), and for Malham Tarn when cladoceran abundance was included as a factor (chl *a*: $p < 0.01$, clado: $p < 0.05$). The mean secchi depth of Malham Tarn may be slightly misleading, as the disc frequently reached the lake bottom, implying theoretical secchi depths exceeding 4 m. Additionally, when weather conditions were adverse and secchi depth could not be measured, it is likely secchi depth was reduced owing to wind-induced sediment resuspension. The true mean may therefore be either lower or higher than 3.55 m.

Seasonal N and P limitation is implied for all three lakes. NO_3^- -N concentrations were below detection limits in Hawes Water and Malham Tarn during most of the growing season; in Malham Tarn beginning in May in 2009, and April in 2010. N limitation is indicated later in the season in Hawes Water, beginning in June in both years. In both lakes, undetectable concentrations persisted to October in 2009, and to the end of the sampling period in September in 2010. In Cunswick Tarn, undetectable concentrations were only recorded once in 2009 (April) and twice in 2010 (June and July). SRP concentrations were frequently low in all lakes, but mostly above detection limits. Concentrations may have decreased to limiting levels, assuming limitation below $3 \mu\text{g L}^{-1}$ as suggested by Reynolds (1998). Using this value, limitation was frequently encountered in Hawes Water and Malham Tarn. In 2009 in both lakes, values were limiting between February and May, and again later in the season, in Hawes Water from August to December, and in Malham Tarn, from September to October. In 2010, limiting values were attained in spring (March - Hawes Water; February, March, May - Malham Tarn) and late summer (August and September - Hawes Water; August - Malham Tarn). P limitation in Cunswick Tarn was infrequent - March, May and June of 2009 only. Seasonally variable, and slightly overlapping, limitation of both P and N is therefore likely in Hawes Water and Malham Tarn through a considerable part of the year, whereas in Cunswick Tarn, limitation is unlikely through most of the year with a higher likelihood in the earlier months of the growing season.

Seasonal anoxia develops in Hawes Water and Cunswick Tarn, but not in Malham Tarn where wind conditions and the shallow water depth eliminate stratification. While chemical stratification in Hawes Water is related to thermal stratification, Cunswick Tarn undergoes chemical

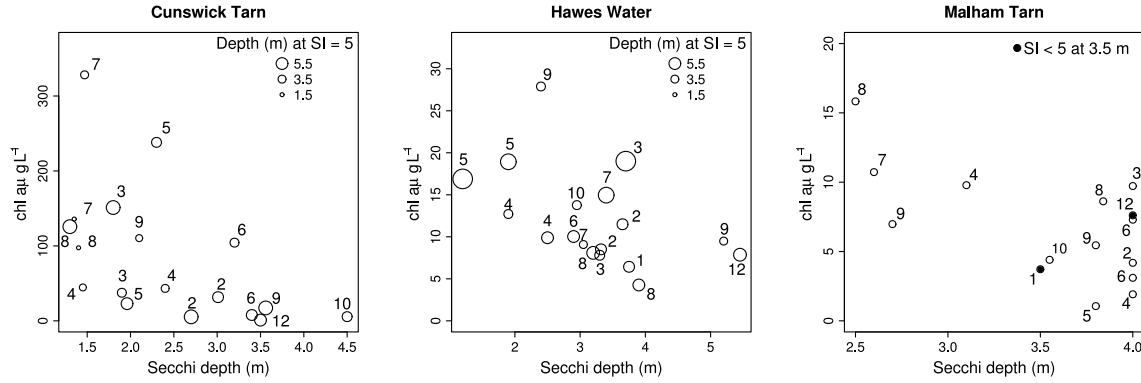


Figure 2.8: Biplots of secchi depth and chlorophyll *a* concentrations for all three lakes. Numbers represent months (1-12), and circle sizes for Cunswick Tarn and Hawes Water reflect the SI 5 boundary depth (Figures 2.11, 2.12). Secchi depth in Malham Tarn truncates at 4m where the disk touches the bottom, and these values do not necessarily reflect true secchi depth.

stratification only. This is reflected in the timing of the development of oxygen gradients, beginning in March in Cunswick Tarn, and May in Hawes Water (Figures 2.9, 2.10). Stratification ends around September. The oxygenated zone in Cunswick Tarn is limited to the uppermost 3m of water, and 6m in Hawes Water. Pockets of supersaturation in Cunswick Tarn coincide with pH maxima (data not shown), reflecting periods of intense photosynthesis. Subsurface oxygen maxima in Hawes Water are highly likely to reflect vertical patchiness of phytoplankton distribution (Reynolds, 1984) (Chapter 3).

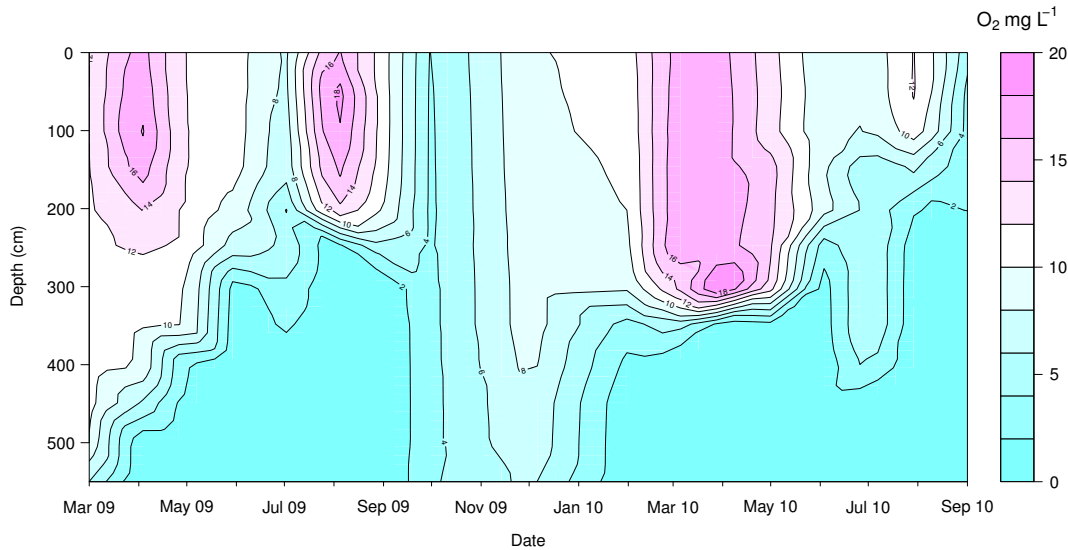


Figure 2.9: The depth profile of oxygen in Cunswick Tarn through years 2009 and 2010.

Light penetration in the lakes is variable with no clear seasonal patterns over the sampling period. Light limitation of macrophyte (charophyte) growth is indicated where light penetra-

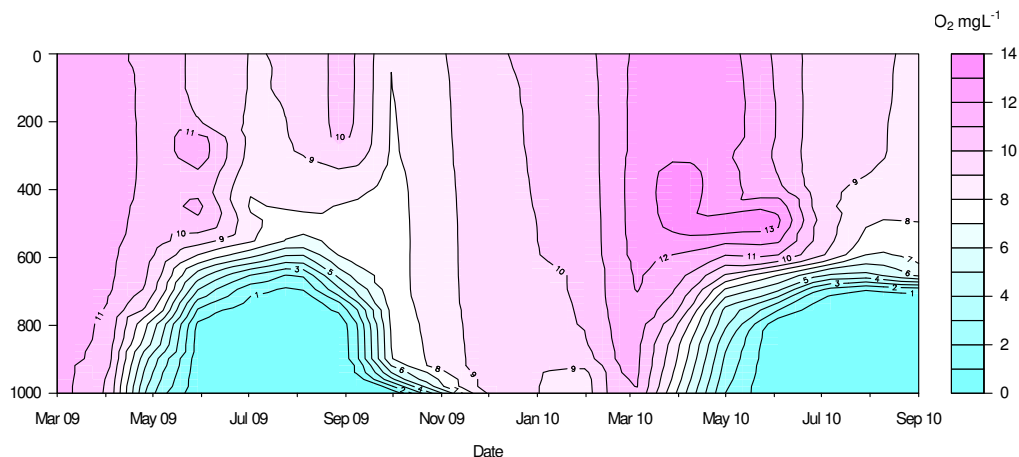


Figure 2.10: The depth profile of oxygen in Hawes Water through years 2009 and 2010.

tion drops to below 5 % of subsurface irradiance (SI), as given by Middelboe and Markager (1997). Using this value as a boundary, seasonal light data reveal theoretical light-limiting conditions in Cunswick Tarn and Hawes Water (Figures 2.11, 2.12), but not for Malham Tarn apart from December 2009 and January 2010 (data not shown). In Hawes Water, the summer (April-September) mean value is 5.5 m, as is the annual mean. In Cunswick Tarn, the summer mean (4 m) is lower than the annual mean (4.3 m). These values are largely in good agreement with observed maximal macrophyte colonisation depths, in Hawes Water and Cunswick Tarn reaching 5 and approximately 4.5 m, respectively. In Malham Tarn, macrophytes grow at all depths. While the relationship between secchi depth and chl *a* in the three lakes is largely negative, the relationship with light penetration in terms of the SI 5 boundary is slightly more ambiguous (Figure 2.8). Large SI 5 depths in Cunswick Tarn and Hawes Water for March 2010 and May 2009 are the consequence of fluctuating incident light conditions leading to a discontinuous light extinction curve, and should not be interpreted as genuine (Figures 2.11, 2.12).

2.4.2 Grazing, predation, and zooplankton-phytoplankton relations

The seasonal dynamics of zooplankton were variable between the lakes, between littoral and open habitats, and between years (data not shown). No overarching, consistent patterns were evident for cladocerans or copepods. Rotifers attained maximal abundance in early summer. In Cunswick Tarn, there was a consistent pattern of high *Daphnia* abundance in June, August and September, with low abundance in July (Figure 2.13), however copepods did not display

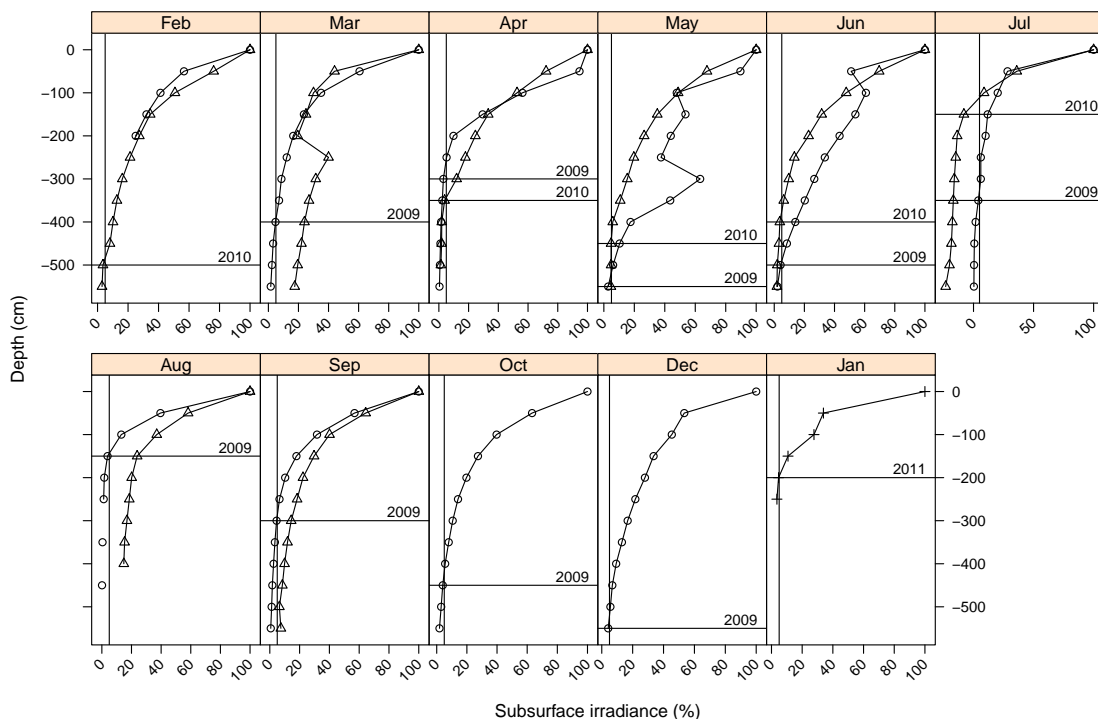


Figure 2.11: Seasonal light penetration data as % subsurface irradiance (SI) for Cunswick Tarn through years 2009 and 2010, and January 2011. Intersecting lines show theoretical depth limits for charophytes assuming a SI of 5 (Middelboe and Markager, 1997). In February and March 2009, and August and September 2010, SI < 5 was below recorded depths. This may be the case for March 2010, as very unstable light conditions prevented standard extinction profiles.

such consistency. Zooplankton densities are in part influenced by the abundance and quality of food (Jeppesen et al., 2011), in part by predators (Carvalho, 1994; Jeppesen et al., 2011), and also by weather patterns (George, 2011), and therefore distribution and abundance can be expected to be very variable. However, differences in the overall zooplankton composition of the three lakes reflect differences in predation pressure on zooplankton.

Cunswick Tarn is fishless as indicated by the complete absence of evidence for fish at present or historically (Chapter 4), and evidence for fish absence such as the high abundance in the plankton of *Chaoborus* spp. including *Chaoborus* cf. *crystallinus/obscuripes* (Luoto and Nevalainen, 2009; Tolonen et al., 2012; Palm et al., 2012). This is reflected in the high abundance of large-bodied *D. hyalina* agg. and *Daphnia pulex*, whereas the smaller *Bosmina* sp. were only found in low abundance in August 2010. Other planktonic species were also rare. Male *Daphnia* were found in early summer (3% of total, May 2010) and more frequently in late summer (around 7% of total; July 2010, August and September both years). Red individuals were common during open water sampling, indicating increased production of haemoglobin

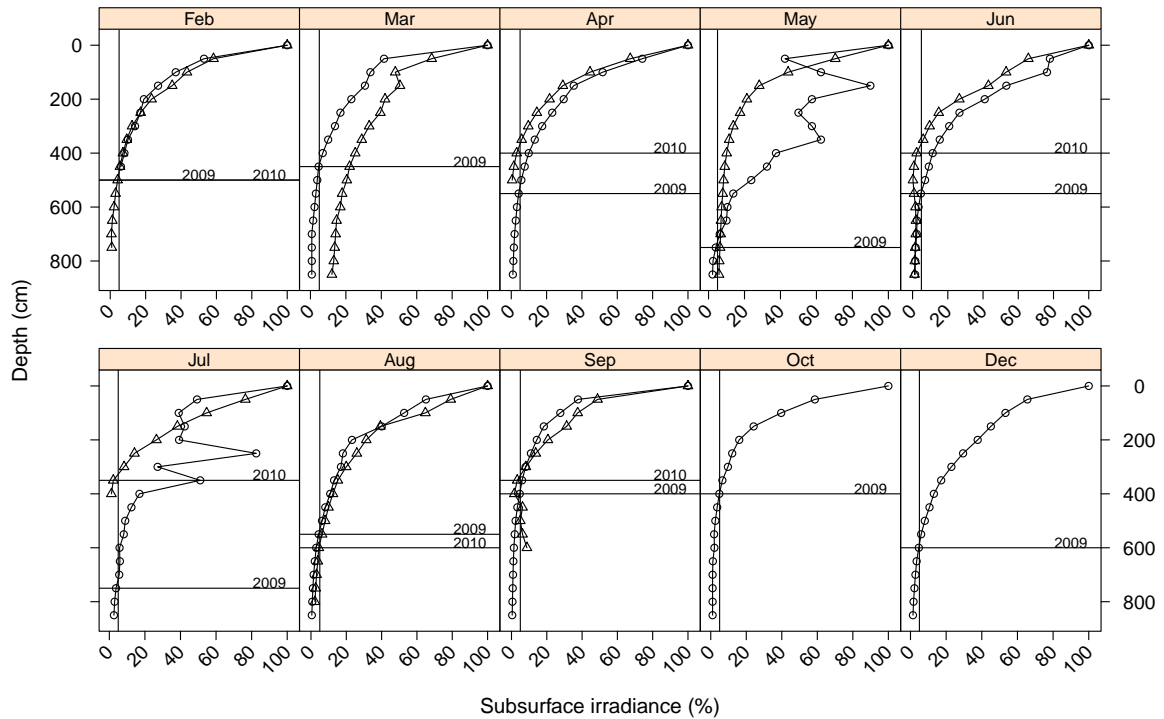


Figure 2.12: Seasonal light penetration data as % subsurface irradiance (SI) for Hawes Water through years 2009 and 2010. Intersecting lines show theoretical depth limits for charophytes assuming a SI of 5 (Middelboe and Markager, 1997). In March and May 2010, SI < 5 was below recorded depths.

under oxygen stress, either owing to vertical migration into the hypolimnion and/or diurnal variation in epilimnetic O_2 . Plant-associated chydorids were dominated by *Acroporus harpae*.

Hawes Water has abundant perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), pike (*Esox lucius*), and tench (*Tinca tinca*) (<http://www.silverdale-cottage.co.uk/the-cottage/fishing/>). *Daphnia* were never found in large numbers, and consisted exclusively of small *D. hyalina* agg. The open-water community was dominated by large numbers of small *Bosmina* sp. (Figure 2.13), with the size of individuals decreasing over summer. *Ceriodaphnia* sp. also appeared in increasing numbers later in summer. The small overall size of pelagic cladocerans, and their decrease in size over summer, reflect high predation pressure (Brooks and Dodson, 1965; Mills et al., 1987; Jeppesen et al., 2011).

Malham Tarn has a diverse fish community including bullhead (*Cottus gobio*), stone loach (*Barbatula barbatula*), three-spined stickleback (*Gasterosteus aculeatus*), minnow (*Phoxinus phoxinus*), perch (*Perca fluviatilis*) and trout (*Salmo trutta*) (Holmes, 1965; Bradley, 2007). The white-clawed crayfish (*Austropotamobius pallipes*) is present in the lake but its abundance

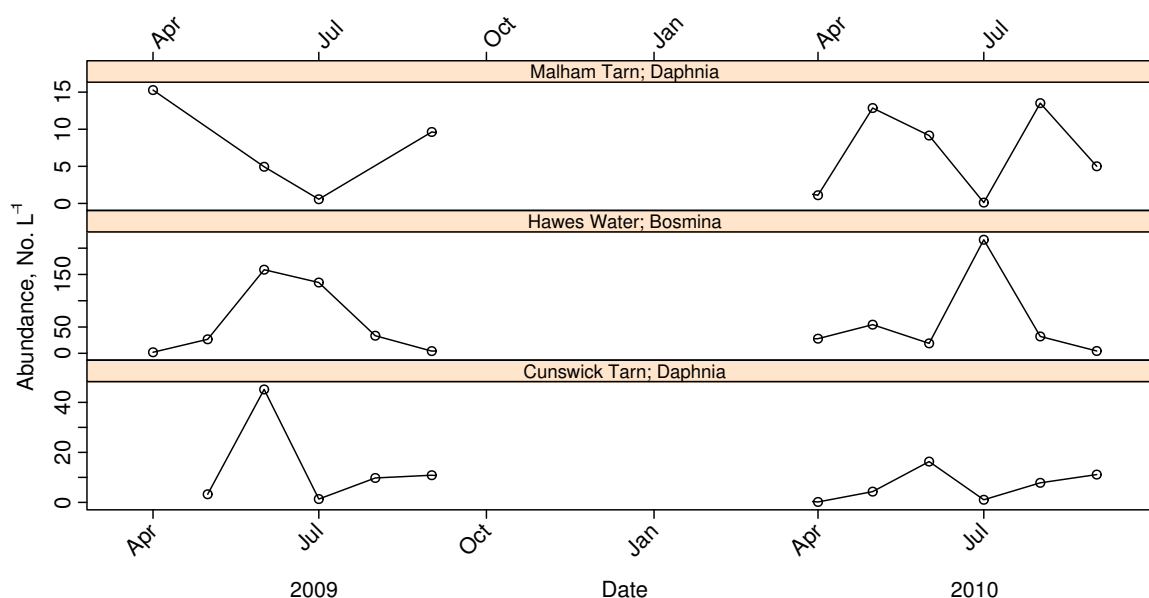


Figure 2.13: Abundance of the dominant pelagic cladoceran genus in Malham Tarn, Hawes Water and Cunswick Tarn in 2009 and 2010.

has decreased dramatically since the 1970s. In contrast to Hawes Water, large-bodied *Daphnia* including *D. pulex* were found throughout summer, in similar abundance to *Bosmina* sp., reflecting decreased predation pressure from zooplanktivores. This may be due to a combination of the presence of piscivorous trout (fishing is catch-and-release and therefore large trout exist in the lake), and shelter provided by macrophyte colonisation throughout the lake, reflected by large catches of chydorids during open water sampling. Male *Daphnia* were found in early summer, in low numbers in April (around 3% of total, both years), and relatively high numbers in May (around 22% of total, both years), and low numbers again in June (3% of total, 2010).

Although variability is evident in the inverse relationship between cladoceran abundance and chl *a*, and the relationship is insignificant for all lakes (Figure 2.14), the latter may be due to the small number of data points. The overall pattern in the relationship in all lakes suggests that cladocerans exert grazing pressure on phytoplankton. The pattern is most obvious for Malham Tarn and Cunswick Tarn, and less so for Hawes Water, where the high cyprinid population is likely to suppress zooplankton and therefore diminish the grazing pressure exerted by zooplankton.

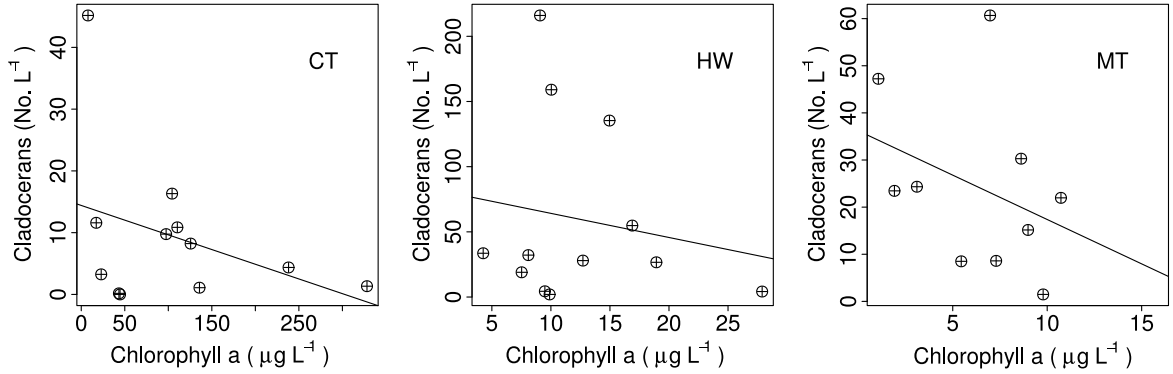


Figure 2.14: Biplots of open water cladoceran density vs chl *a* concentrations. Simple regression lines on raw data serve a visual purpose only.

2.4.3 Present and past macrophyte communities

General patterns of abundance, richness, and seasonality

All three lakes have full macrophyte cover where light conditions and sediment structure are favourable, leaving no physically suitable habitat uncolonised. In Malham Tarn, macrophytes cover all areas apart from exposed rocky shores (where filamentous algal strands and *Fontinalis antipyretica* are occasional). In Hawes Water and Cunswick Tarn, macrophyte coverage extends to water depths of approximately 5 m and 4.5 m, respectively. It follows that the pelagic zones in Cunswick Tarn and Hawes Water are extensive.

Submerged and floating-leaved macrophyte species richness, excluding structurally complex filamentous algae, as recorded over the period 2009 - 2010 is greatest for Hawes Water (15) and Malham Tarn (14), and poorest for Cunswick Tarn (4) (Tables 2.5, 2.6, 2.7). While Cunswick Tarn has no charophytes at present, there are several species in Hawes Water (4) and Malham Tarn (6). The *Potamogeton* assemblage is poorer, with three species in Malham Tarn, two in Hawes Water, and one species in Cunswick Tarn (Tables 2.5, 2.6, 2.7). Emergent macrophytes (4 spp.) are most dense and abundant in Hawes Water, dominating the lake margins especially to the west and forming a continuous reedswamp all around interspersed with *Alnus* and *Salix*. Emergents are less abundant in Cunswick Tarn (3 spp.), where they grow as relatively narrow bands and *Cladium mariscus* only grows as one patch in the northwest. There are large *Salix* around the lake margins, and macrophyte cover under their branches is reduced. Malham Tarn has only two patches of emergent vegetation (predominantly *Carex rostrata*), one at the northwest end by the inflow, and one in a small bay in the northeast

Table 2.4: Depth distribution (cm) of macrophytes in the study sites at each transect in 2009 and 2010. Depth_{max} & Depth_{maxcol} refer to maximal depth surveyed, and maximal depth of colonisation, respectively. The species found at the maximal colonisation depth are; cglob = *Chara globularis*, cglvir = *Chara globularis/virgata* intermediate, ecan = *Elodea canadensis*, fanti = *Fontinalis antipyretica*, nflex = *Nitella flexilis* agg., nopa = *Nitella opaca*, nlut = *Nuphar lutea*, ppus = *Potamogeton pusillus/berchtoldii*

June 2009					August 2009		
Site	Transect	Depth _{max}	Depth _{maxcol}	Species	Depth _{max}	Depth _{maxcol}	Species
CT	1	434	391	ecan	574	410	ecan, ppus
	2	450	450	ecan, ppus	476	370	ecan
MT	1	310	310	cglob, nflex	320	320	cglob
	2	250	250	nopa, cglob	280	280	cglob, nflex
	3	360	360	fant, cglob	340	340	cglob
	4	370	340	fant, nopa	NA	NA	NA
HW	1	420	420	nlut	550	468	nlut
	2	500	500	nlut	465	465	nlut
	3	400	400	pluc, nlut	530	410	pluc, nlut
	4	310	310	nlut	415	415	nlut

June 2010					August 2010		
Site	Transect	Depth _{max}	Depth _{maxcol}	Species	Depth _{max}	Depth _{maxcol}	Species
CT	1	543	403	ecan	NA	NA	NA
	2	434	391	ecan	NA	NA	NA
MT	1	280	280	cglvir	245	245	nflex, ecan, cglvir
	2	275	275	ecan, nopa, cglvir	225	225	ecan, nflex
	3	400	400	nopa, fant	350	350	nflex
	4	395	395	cglvir, nopa	375	375	cglvir
HW	1	553	473	nlut	425	425	nlut
	2	397	397	nlut	435	435	nlut
	3	415	415	nlut	450	450	nlut
	4	535	475	nlut	525	440	nlut

corner, where sediments are silty and wind stress is reduced. Filamentous algae are locally abundant in Cunswick Tarn, as dense, floating mats of *Cladophora* sp. in June/July. In Malham Tarn and Hawes Water, filamentous algae are found in shallow water coating rocks and macrophytes. In Malham Tarn, growth is abundant to the extent of smothering other vegetation by net-like overgrowth by the inflow as well as the outflow.

Patterns of macrophyte seasonality are different between the sites. Abundant macrophyte growth has been observed in Malham Tarn through October to the end of November (*Chara globularis*, *Nitella flexilis* agg., *Callitriche hamulata* agg., *Elodea canadensis*, *Potamogeton crispus*, *Potamogeton lucens*, *Zannichellia palustris*), and charophytes overwinter. *Chara ac-*

Table 2.5: Historical records of macrophytes in Malham Tarn, and all species recorded 2009/2010. The growth form of *Aegagropila linnaei* is the globally rare ball (Lund, 1961; Boedeker et al., 2010). Annual (August) surveys of the Tarn have also been conducted by Hinton (2005).

Date	Flora	Source
2009,2010	<i>Aegagropila linnaei</i> , <i>Chara aspera</i> , <i>Chara globularis</i> , <i>Chara virgata</i> , <i>Nitella opaca</i> (<i>Nitella flexilis</i>), <i>Fontinalis antipyretica</i> , <i>Callitriche hamulata</i> , <i>Elodea canadensis</i> , <i>Equisetum fluviatile</i> , <i>Lemna minor</i> , <i>Menyanthes trifoliata</i> , <i>Potamogeton berchtoldii</i> , <i>Potamogeton crispus</i> , <i>Potamogeton lucens</i> , <i>Zannichellia palustris</i> + <i>Hippuris vulgaris</i> as strandline wash	Surveys by author
2005	<i>C. aspera</i> , <i>C. globularis</i> , <i>C. virgata</i> , <i>N. flexilis</i> agg., <i>N. opaca</i> , <i>Tolypella glomerata</i> , <i>F. antipyretica</i> , <i>C. hamulata</i> , <i>Callitriche</i> cf. <i>platycarpa</i> , <i>E. canadensis</i> , <i>L. minor</i> , <i>M. trifoliata</i> , <i>P. berchtoldii</i> , <i>P. lucens</i> , <i>P. crispus</i> , <i>Z. palustris</i>	Survey by B. Goldsmith and A. Burgess, ENSIS
1995	<i>A. linnaei</i> , <i>C. globularis</i> , <i>F. antipyretica</i> , <i>C. hamulata</i> , <i>E. canadensis</i> , <i>M. trifoliata</i> , <i>P. crispus</i> , <i>P. lucens</i>	Bennion et al. (1997)
1993	<i>Chara</i> spp. and <i>M. spicatum</i> abundant, too early in season for <i>P. lucens</i>	Richardson and Magee (1995)
1960s	<i>A. linnaei</i> , <i>C. aspera</i> , <i>C. globularis</i> , <i>F. antipyretica</i> , <i>E. canadensis</i> (1962, Rare), <i>M. spicatum</i> , <i>P. berchtoldii</i> , <i>P. lucens</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton gramineus</i> x <i>perfoliatus</i>	Lund (1961); Holmes (1965), J. Birks, school trip
1950s	<i>A. linnaei</i> , <i>C. aspera</i> , <i>C. globularis</i> , <i>F. antipyretica</i> , <i>H. vulgaris</i> , <i>M. spicatum</i> , <i>P. berchtoldii</i> , <i>P. lucens</i> , <i>P. natans</i> , <i>P. perfoliatus</i> , <i>P. gramineus</i> x <i>perfoliatus</i> , <i>P. polygonifolius</i>	Various herbaria collectors (herbariaunited.org), Pigott and Pigott (1959); Holmes (1956, 1965)
1936	<i>C. aspera</i> , <i>C. globularis</i> , <i>F. antipyretica</i> [“plentiful in the region round the inlet”], <i>Hypnum scorpioides</i> , <i>Callitriche stagnalis</i> [“in the shallow western bay”], <i>Littorella uniflora</i> [northeast corner], <i>M. spicatum</i> , <i>P. alpinus</i> [by boathouse], <i>P. lucens</i> [“locally dominant forming dense beds off the southern and eastern shores”], <i>P. perfoliatus</i> ; Colonisation through all depth zones, but “much of the central and western part is devoid of bottom vegetation”	Sledge (1936)
1925	“Examining the pondweeds washed up along the edge of the Tarn...”: <i>P. x angustifolius</i> (<i>x zizii</i>), <i>P. lucens</i> , <i>P. lucens</i> var. <i>acuminatus</i> , <i>P. perfoliatus</i> , <i>P. praelongus</i>	Pearsall and Mason (1925)
1741	“There are no weeds in it. In a fine still day, you may see the white chalky bottom, where it is 10 or 12 foot deep.”	Fuller (1741)

Table 2.6: Historical records of macrophytes in Hawes Water, and all species recorded 2009/2010.

Date	Flora	Source
2009 & -10	<i>Chara aculeolata</i> , <i>Chara aspera</i> , <i>Chara contraria</i> , <i>Chara virgata</i> , <i>Fontinalis antipyretica</i> , <i>Cladium mariscus</i> , <i>Elodea canadensis</i> , <i>Hippuris vulgaris</i> , <i>Lemna minor</i> , <i>Lemna trisulca</i> , <i>Myriophyllum spicatum</i> , <i>Nuphar lutea</i> , <i>Nymphaea alba</i> , <i>Phragmites australis</i> , <i>Potamogeton coloratus</i> , <i>Potamogeton lucens</i> , <i>Schoenoplectus lacustris</i> , <i>Typha angustifolia</i> , <i>Utricularia vulgaris</i> agg.	Surveys by author
1999	<i>C. hispida</i> var. <i>hispida</i> (syn. <i>C. aculeolata</i>), <i>C. hispida</i> var. <i>major</i> ; <i>Chara rudis</i> to 4m depth; <i>P. lucens</i>	Survey by C. Newbold
1995	<i>C. aculeolata</i> , <i>C. aspera</i> , <i>Chara curta</i> , <i>C. hispida</i> , <i>C. rudis</i> , <i>P. lucens</i> , <i>Potamogeton natans</i>	1996 Natural England report for Cumbria County Council and ARC
1993	<i>Chara</i> sp. recorded at HAWE3 coring site at depths around 4 m	Field notes by J. Marshall
1982	encrusted <i>C. aspera</i> abundant, <i>C. rudis</i> to 7 m depth; <i>E. canadensis</i> , <i>P. lucens</i> ; “water was very brown in colour and visibility was poor.” - M. Wade	Diver survey by C. Newbold et al.
1969	<i>C. aculeolata</i> , <i>C. aspera</i> , <i>C. mariscus</i> , <i>N. lutea</i> , <i>N. alba</i> , <i>P. australis</i> , <i>Potamogeton crispus</i> , <i>Potamogeton friesii</i> , <i>P. lucens</i> , <i>Potamogeton obtusifolius</i>	School trip; J. Birks
1915	<i>C. aculeolata</i> , <i>Chara fragilis</i> subsp. <i>delicula</i> (possibly syn. <i>C. globularis</i>), <i>C. rudis</i> , <i>C. vulgaris</i> var. <i>papillata</i> , <i>P. friesii</i> , <i>P. lucens</i> , <i>P. obtusifolius</i> , <i>H. vulgaris</i> , <i>N. lutea</i> , <i>N. alba</i> ; “The Characeae are in considerable quantity and much encrusted”	W. H. Pearsall, in Druce (1916)
1911	A record of <i>Littorella uniflora</i>	Druce (1911)

uleolata and *Potamogeton coloratus* in Hawes Water are perennial, but other species die back in autumn. Dieback in Cunswick Tarn is comparatively early in the season, occurring around September/October, although *E. canadensis* maintains viable underwater turions through winter that begin to grow in February.

Species composition and distribution

Table 2.7: Historical records of macrophytes in Cunswick Tarn (substantial proportion of data originally supplied by J. Ogden, Natural England), and all species recorded 2009/2010.

Date	Flora	Source
2009 & -10	<i>Elodea canadensis</i> , <i>Cladium mariscus</i> , <i>Nuphar lutea</i> , <i>Nymphaea alba</i> , <i>Phragmites australis</i> , <i>Potamogeton pusillus</i> agg., <i>Schoenoplectus lacustris</i>	Surveys by author 2009/2010
2004	<i>N. lutea</i> , <i>Potamogeton obtusifolius</i> , <i>P. pusillus</i>	Darwell (2004)
1983	<i>Chara hispida</i> , <i>Nitella opaca</i> , <i>Carex elata</i> , <i>C. mariscus</i> , <i>E. canadensis</i> , <i>Equisetum fluviatile</i> , <i>Iris pseudacorus</i> , <i>Myriophyllum</i> sp., <i>N. lutea</i> , <i>N. alba</i> , <i>P. australis</i> , <i>Potamogeton natans</i> , <i>P. obtusifolius</i> , <i>P. pusillus</i> , <i>S. lacustris</i> , <i>Sparganium minimum</i>	“Lakes in Cumbria” survey by E. Charter
1982	<i>Carex rostrata</i> , <i>C. mariscus</i> , <i>E. fluviatile</i> , <i>Hydrocotyle vulgaris</i> , <i>I. pseudacorus</i> , <i>Menyanthes trifoliata</i> , <i>N. lutea</i> , <i>N. alba</i> , <i>Phalaris arundinacea</i> , <i>P. australis</i> , <i>Potentilla palustris</i> , <i>S. lacustris</i>	Survey by E. Charter and H. Frankland
1977 & -80	<i>C. hispida</i> , <i>N. opaca</i> , <i>C. mariscus</i> , <i>E. canadensis</i> , <i>E. fluviatile</i> , <i>I. pseudacorus</i> , <i>P. australis</i> , <i>N. lutea</i> , <i>N. alba</i> , <i>S. lacustris</i> ,	Stokoe (1983)
1969	<i>Chara aculeolata</i> , <i>C. mariscus</i> , <i>N. lutea</i> , <i>P. australis</i>	School trip; J. Birks
1954	” <i>Cladium mariscus</i> , east side dominated by <i>Phragmites</i> which appears to be spreading”	Lewis (1954)
1950	”Excellent calcareous tarn with <i>Cladium mariscus</i> and interesting sedges”	NC SSSI citation, W. H. Pearsall
1938	<i>C. aculeolata</i> , <i>C. desmacantha</i> [syn <i>C. curta</i>], <i>Chara fragilis</i> [syn. <i>C. globularis</i>]; “very rich in aquatic plants, including the large sedge <i>Cladium mariscus</i> ”	Wilson (1938)
1899	<i>Chara curta</i>	Stewart (2001)

The macrophyte community in Cunswick Tarn is dominated by *Nuphar lutea* and *E. canadensis*. *Nymphaea alba* and *Potamogeton pusillus/berchtoldii* exist as patches. *Cladophora* sp. is most abundant in the shallows by the north shore where grain is deposited in summer as feed for waterfowl. *N. lutea* extends mostly to depths of 3-3.5 m, whereas *E. canadensis* and *P. pusillus/berchtoldii* occupy the deeper zones (Table 2.4). Hawes Water also displays vertical zonation, although in contrast to Cunswick Tarn, Nymphaeaceae typically dominate

Table 2.8: Species lost in each lake. Some old records of *Chara* may be problematic due to changes in taxonomy and nomenclature over time. *Potamogeton gramineus*, previously in Malham Tarn as the hybrid with *Potamogeton perfoliatus*, can still be found down the outflow.

Lake	No. lost	Species
Cunswick Tarn	9	<i>Chara aculeolata</i> , <i>Chara curta</i> (syn. <i>Chara aspera</i> ?), <i>Chara globularis</i> , <i>Chara hispida</i> , <i>Nitella opaca</i> , <i>Myriophyllum spicatum</i> , <i>Potamogeton natans</i> , <i>Potamogeton obtusifolius</i> , <i>Potamogeton praelongus</i>
Hawes Water	6 (7?)	<i>Chara rudis</i> , <i>Littorella uniflora</i> , <i>Potamogeton crispus</i> , <i>Potamogeton friesii</i> , <i>Potamogeton natans</i> , <i>Potamogeton obtusifolius</i> , possibly <i>Potamogeton perfoliatus</i>
Malham Tarn	9	<i>Littorella uniflora</i> , <i>Myriophyllum spicatum</i> , <i>Potamogeton alpinus</i> , <i>Potamogeton gramineus</i> \times <i>perfoliatus</i> , <i>Potamogeton natans</i> , <i>Potamogeton perfoliatus</i> , <i>Potamogeton praelongus</i> , <i>Potamogeton polygonifolius</i> , <i>Potamogeton</i> \times <i>zizii</i>

the deepest colonised zone (Table 2.4). Shallow water is dominated by the emergents *Typha angustifolia*, *C. mariscus* and *Phragmites australis*, and also by *Utricularia vulgaris* agg. and *P. coloratus*. Beyond a depth of 75 cm, *Schoenoplectus lacustris*, *N. lutea* and *C. aculeolata* are abundant. Depths exceeding 2 m are almost exclusively colonised by *N. lutea*. *P. lucens* and *H. vulgaris* are found as patches to the south and southeast, and to the west, respectively. There is a patch of an ornamental nymphaeid in the western margin. The charophyte species recorded as rare (*Chara aspera*, *Chara contraria*, *Chara virgata*) are confined to shallow areas where there are gaps in the reedswamp.

Shallow areas in Malham Tarn are for the most part colonised by *C. hamulata* agg., *Fontinalis antipyretica* and filamentous algae to the north. To the south and east, *C. aspera* grows as short tufts. By the outflow, *E. canadensis*, *Potamogeton crispus* and filamentous algae are particularly abundant. The east margin has very little macrophyte development by the rocky shoreline, with occasional growth of *Z. palustris*. Deeper water (>2 m) is dominated by *E. canadensis*, *Nitella opaca* (*Nitella flexilis*) agg. and *C. globularis*, and also to the north by *P. lucens* and *Fontinalis antipyretica* (Table 2.4).

Centennial-scale changes in macrophyte composition

All three lakes had a more diverse macrophyte community in the first half of the 20th century compared with the present day, and species lost belong mostly to the Characeae and Potamogetonaceae (Table 2.8). In Hawes Water, species have been lost from all macrophyte

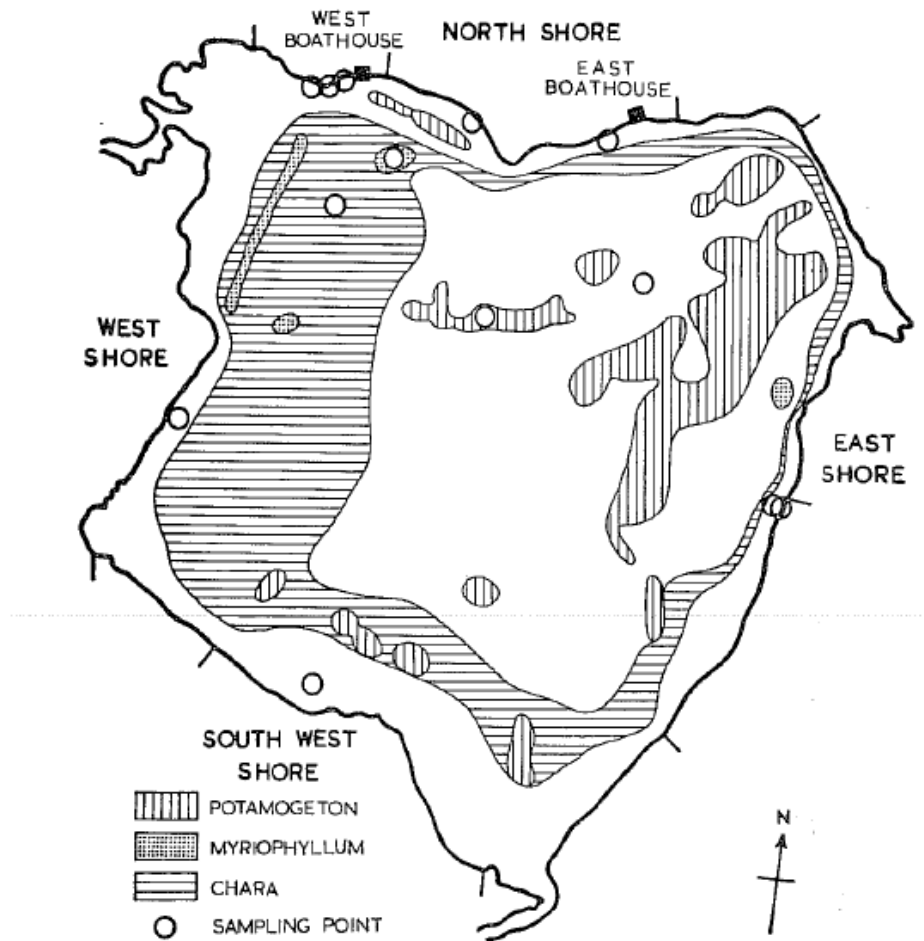


Figure 2.15: Macrophyte coverage in Malham Tarn in 1968, from Philipson (1968). White areas are bare sediment.

zones, with *Littorella uniflora* from the littoral, *P. berchtoldii* agg., *P. crispus*, *Potamogeton friesii*, *Potamogeton natans*, *Potamogeton obtusifolius* from intermediate depths, and *Chara rudis* from depths of at least 7 m. The extirpation of *C. rudis* is directly related to decreasing maximal colonisation depth (Chapter 3, Table 2.6). Owing to changes in nomenclature, it is impossible to produce an exact number of extirpated *Chara* species. However, it is also likely that *C. globularis* (another deeper-growing species), *Chara vulgaris* var. *papillata* and *Chara hispida* var. *major* are separate from species recorded currently. Further, the distribution of remaining charophytes has become increasingly restricted. *C. aspera* was abundant in the 1980s whereas it was only occasional during 2009/2010. Despite extensive searches in the reedswamp, including areas where it had been recorded in 2009, *C. virgata* was not found in 2010. Other species are also likely to have been patchily distributed, for example *C. curta* was only recorded in 1995. Owing to the extensive reedswamp, complete accounts of charophyte

species are unlikely, both historically and presently.

Mapped species distributions further show that the distributions of *E. canadensis*, *Hippuris vulgaris* and *P. lucens* have shrunk since the 1980s, whereas palaeolimnological data indicate an increase in the abundance of Nymphaeaceae over the same time period (Chapter 3).

Cunswick Tarn has lost all charophyte species, with the last records in 1983 including *C. hispida* and *N. opaca* (Table 2.7). The last record of *C. aculeolata* dates from 1969, and descriptions of classic marl lake communities date from the early 1900s to the 1950s. The description of “rich” plant growth in the 1930s is in excellent agreement with the fossil record, indicating a mixture of Nymphaeaceae, Characeae and Potamogetonaceae. However, even higher abundance of charophytes is indicated in the early 1900s through high abundances of fossil oospores of varying morphology. It is highly likely that changes in light penetration also extirpated charophytes from Cunswick Tarn (Chapter 4). Of the angiosperms, *P. obtusifolius* was last recorded in 2004, and *M. spicatum* and *P. natans* in 1983. The fossil record also indicates *Potamogeton praelongus* presence in Cunswick Tarn, with one seed found at a depth corresponding to the early 1900s, and one to the pre-1800s. It is likely this species disappeared from the lake relatively early, owing to absence also from the relatively comprehensive surveys over the 1970s and 1980s.

Malham Tarn has not experienced a reduction in colonisation depth, nor have charophyte species disappeared from the lake. However, seven *Potamogeton* species have disappeared over the 20th century, namely *Potamogeton alpinus*, *P. natans*, *Potamogeton polygonifolius*, *Potamogeton gramineus* *x* *perfoliatus*, *P. perfoliatus*, *P. praelongus* and *Potamogeton* *x* *zizii*. *P. gramineus* can still be found down the outflow channel in the southeast. As for Hawes Water, *L. uniflora* is likely to have been lost relatively early, as the only record of the species dates from 1936. Further, *Myriophyllum spicatum* has been greatly reduced in abundance. It was recorded in several patches in the 1960s (Holmes (1965), Figure 2.15) and only rarely in the surveys by G. Hinton. None were found in 2009 and 2010 and the species is tentatively claimed to be lost from the community. Accounts of overall macrophyte abundance indicate a large increase in % macrophyte cover over time. In 1741, a “white chalky bottom” was described to depths exceeding 3 m. Equally, in 1936, macrophyte coverage was not complete despite colonisation to the highest depth (Sledge, 1936), and relatively low cover was also observed in 1968 (Figure 2.15) compared with the present (approximately 100% available

substrate). Over the last few decades, large interannual variability (cyclicality) in macrophyte coverage, involving reciprocal fluctuations of *C. globularis* and *E. canadensis*, has been observed (Hinton, 2005), with lows of less than 40% (G. Hinton, pers. comm.). Field data from 2009 and 2010 may therefore reflect the current rising trend in cover.

2.4.4 WFD and CSM classifications

For Malham Tarn, the simple indices of TP ($18 \mu\text{g L}^{-1}$), chl *a* EQR (0.90) and macrophyte colonisation depth ($>4\text{ m}$) indicate that it has the highest ecological status of the study sites. The scores for each index (G, H, H) suggest Good or High ecological status at present. Hawes Water is more impacted, with TP ($20 \mu\text{g L}^{-1}$), chl *a* EQR (0.65), and colonisation depth (5 m) indicating Good/Moderate, Good, and Moderate ecological status, respectively. The scores are lowest for Cunswick Tarn, with TP ($56 \mu\text{g L}^{-1}$), chl *a* EQR (0.22), colonisation depth (4.5 m) yielding Poor, Poor and Moderate, respectively.

In terms of CSM, Cunswick Tarn failed due to absence of characteristic species (charophytes), high abundance of *E. canadensis*, and high TP. Owing to the permanent loss of characteristic species, the site can be designated to destroyed status. Through the inclusion of palaeolimnological and historical evidence, Hawes Water is also classed unfavourable due a reduction in maximal plant colonisation depth and the loss of the characteristic species *Chara rudis*. The mean TP ($20 \mu\text{g L}^{-1}$) is also at the unfavourable/favourable boundary and could warrant unfavourable status. Malham Tarn stands alone in favourable status, although the periodic dominance of *E. canadensis* would add the qualifier “at risk”.

Macrophyte community indices yield variable statuses for all three lakes at present (Table 2.10). Malham Tarn and Hawes Water have identical scores (H) for all but LMNI, placing Malham Tarn in the Good, and Hawes Water in the High category. Through the rule-based weighting of the indices, this difference results in designating Malham Tarn as Good, and Hawes Water as Moderate. Cunswick Tarn has relatively low filamentous algal cover, and high representation of each species present, yielding High status, however LMNI, N_TAXA and N_FG all yield Moderate. These indices take precedence and result in Moderate classification.

Historically, LMNI EQRs indicate a reduction in status over time for Cunswick Tarn (H \rightarrow M) and Malham Tarn (H \rightarrow G), in contrast to Hawes Water that, owing to a peculiar set of

Table 2.9: Macrophyte-based EQRs and resulting status bins for CT, HW and MT; the final adjusted EQR and resulting WFD classification has been calculated as per (UKTAG 2012) (see Methods)

Site	LMNI	N_FG	N_TAXA	COV	ALG	Status	Past Status
Cunswick Tarn	.54 (M)	.54 (M)	.50 (M)	1 (H)	1 (H)	.42 Moderate	.89 High
Hawes Water	.88 (H)	1 (H)	1 (H)	.81 (H)	1 (H)	.85 High	.89 High
Malham Tarn	.78 (G)	1 (H)	1 (H)	.87 (H)	1 (H)	.78 Good	.98 High

Table 2.10: Simple indices and resulting status bins, and CSM status, for CT, HW and MT; chl *a* EQR and resulting WFD classification has been calculated as per (UKTAG 2012) (see Methods)

Site	Chl <i>a</i> EQR	TP ($\mu\text{g L}^{-1}$)	Z _{col} (m)	CSM status
Cunswick Tarn	.22 (P)	56 (P)	4.5 (M)	destroyed
Hawes Water	.65 (G)	20 (G/M)	5 (M)	unfavourable
Malham Tarn	.90 (H)	18 (G)	4 (H)	favourable (at risk)

compositional changes, has near-identical EQRs for both the early 1900s and 2009/2010 (H \rightarrow H).

2.5 Discussion

2.5.1 The ecological status of Malham Tarn, and implications for very shallow marl lakes

The ecological status of Malham Tarn by WFD methodology is Good, owing to the single Good score of the LMNI receiving high weight. All other indices indicate High status, and CSM attributes also rank Malham Tarn in favourable status. Historical and observational data suggest, however, that Malham Tarn is not in High ecological status. This is based primarily on assessment of the macrophyte communities, but there are also accounts of changes in the plankton (Talling and Parker, 2002) and sedimentation rate (Bradley, 2007). Among the macrophytes, there have been changes both in species composition and distribution. Several *Potamogeton* spp. including *P. polygonifolius*, *P. praelongus*, *P. perfoliatus*, *P. gramineus* *x* *perfoliatus* and *P. natans* disappeared from the lake between the 1930s and the 21st century, the first two in particular being representative of unimpacted lakes (Sand-Jensen et al., 2000; Willby, 2005). While *P. natans* is associated with lakes in variable status, the species has also been shown to decline with eutrophication (Egertson et al., 2004), having disappeared also from Cunswick Tarn. In contrast, the currently locally abundant *P. crispus* and *P. berchtoldii* are associated with eutrophic habitats (see e.g. Sand-Jensen et al., 2000; Davidson et al., 2008;

Bennion et al., 2009; Willby et al., 2010). Records of *Zannichellia palustris* and *Callitriche* sp. both by the inflow, but *Z. palustris* also in the east margin, since 2005, are more recent signs of eutrophication (Willby, 2005). Importantly, these changes have preceded serious reductions in light penetration, and show relatively slow yet sustained decline in ecological status.

The effect of deteriorating light conditions on charophyte meadows in Malham Tarn is retarded by the prevailing shallow water depths, as indicated by secchi depths reaching the lake bottom and <5% SI only recorded in winter, reflecting stormy weather and sediment resuspension rather than phytoplankton blooms (Figure 2.6). However, the relative absence of the latter (although observations suggest phytoplankton have become more abundant; Bradley 2007) does not prove absence of stress on macrophytes. Epiphytes also induce light limitation for macrophytes, and increases in epiphytes have been suggested to precede shifts towards more pelagic productivity (Phillips et al., 1978; Moss, 1978; Sayer et al., 2010), especially where fish exert variable cascade pressure (Jones and Sayer, 2003) and wind is not significant (Strand and Weisner, 2001). Palaeolimnological data also indicate that an increase in epiphytes preceded macrophytic-pelagic transitions in Cunswick Tarn through the early- mid- 1900s (Chapter 4). While macrophytes in exposed areas of Malham Tarn are free of epiphytic filamentous algae, in more sheltered areas by the inflow as well as the outflow, they are periodically smothered, suggesting that a similar transition may be under way. Further, while macrophytes at the outflow grow under the algae, reaching close to the water surface, macrophytes at the inflow are in poor health, stunted and completely covered, with forests of filamentous algae covering the benthos. It is highly likely that, with less wind exposure, the deeper-growing macrophyte communities would also be affected. This is in stark contrast to the lake in the 1960s and 1970s, when *Myriophyllum spicatum* (now rarely found if at all) grew by the inflow (Philipson, 1968; Calow, 1973), suggesting substantial changes by the inflow area.

Chl *a*, TP and colonisation depth do not capture the aforementioned changes owing to the (so far) reduced effects of eutrophication in the pelagic zone, and the overall shallow depth of Malham Tarn. In very shallow lakes, macrophytes are expected to account for the majority of in-lake productivity, suppressing phytoplankton biomass, and therefore pelagic indices do not show change relating to % macrophyte cover or species succession occurring in the benthos (Canfield Jr. et al., 1983). It was initially hypothesised that macrophyte-based indices would be better able than water column indices to reflect status as implied by observational and

historical data. This prediction was partly met, however, owing to the high number of taxa currently present in the lake, species and functional group richness indices place Malham Tarn in High status. LMNI gives the lowest ranking of Good status. This relatively low ranking may either be a reflection of the weakness of the index for sites with high diversity (Willby et al., 2010) or in contrast, be successful at capturing degradation that other indices do not. The latter is more likely, as change in species composition over time is reflected in LMNI, with High status indicated for both the 1920s/1930s and 1950s when several of the Potamogetonaceae now absent were present, and species associated with eutrophication such as *E. canadensis*, *P. crispus*, *Z. palustris* were not recorded. This is also apparent when comparing present macrophyte communities with expected species abundances along the ecological quality gradient (Table 2.11).

Based on data for past communities and thorough surveying of Malham Tarn, it is argued here that the site is neither of High/Good nor favourable status. Rather, as indicated by the species loss, appearance of eutrophic species, and increasingly homogenous composition of macrophytes, combined with the expansion of filamentous algae, the current condition of the lake would appear to be Moderate. Therefore, it is argued that chl *a* and supporting TP boundaries for very shallow marl lakes should be more stringent, in agreement with the boundaries suggested by Free et al. (2007), which would place Malham Tarn at Moderate status. The classification of Malham Tarn into favourable status through CSM reflects the prevalence of charophytes on the site. The inclusion of associate species, and their community composition changes, in CSM assessment, would likely have resulted in unfavourable status.

Importantly, as discussed by Willby et al. (2010), high macrophyte coverage and species richness may not indicate good status where reference conditions involve nutrient limitation and therefore relatively low macrophyte biomass and potentially low diversity (Dodson et al., 2000). This is in contrast to lakes under higher nutrient loading where biodiversity does decline with increasing nutrient concentrations (Jeppesen et al., 2000). High diversity may also be expected under conditions of high light penetration, in part disconnecting diversity patterns from TP (Vestergaard and Sand-Jensen, 2000). In Malham Tarn, the high coverage and diversity may be a reflection of the increase expected under early stages of eutrophication, as earlier records indicate lower % coverage (Sledge, 1936; Philipson, 1968) and “chalky bottoms” exceeding water depths of 3 m (Fuller, 1741), a depth zone which is currently heav-

ily colonised and has fine sediments. Unfortunately a palaeolimnological study of Malham Tarn was not conducted as part of the current study and therefore ecological shifts have not been determined. However several other studies indicate enrichment over several centuries (Pentecost, 1998; Bradley, 2007).

2.5.2 The ecological status of Hawes Water, and implications for shallow marl lakes

The simple indices TP, chl *a* and macrophyte colonisation depth indicate Good, Good/Moderate, and Moderate status for Hawes Water. In contrast, and similarly to Malham Tarn, WFD macrophyte indices score higher, all indicating High status. Through the one-out-all-out WFD rule, chl *a* places Hawes Water in Good status. Observational and historical data suggest Hawes Water is relatively heavily impacted, both in terms of species loss and reductions in colonisation depth related to increases in the plankton, reflecting as unfavourable status under CSM.

It is interesting that the historical macrophyte communities (1900-1930s) receive the same score as current communities using the LMNI index, as substantial changes in macrophyte composition have occurred. For example, 4-5 *Potamogeton* species recorded historically disappeared from the site between the 1960s and 1980s, and the distribution of *P. lucens* at present (S/SE margins) is greatly reduced compared with the 1980s when surveys showed patches also at the north end. These changes are associated with a substantial increase in the floating-leaved Nymphaeaceae (largely *N. lutea*) as indicated by the fossil record (Chapter 3). In the shallower littoral zone, *L. uniflora* disappeared relatively early (no record since 1911), and charophyte species recorded as Rare are restricted to the relatively few areas of open water in the reedswamp. Historical photographs (from ground and aerial) indicate that the reedswamp may have become denser with time, in support of which macrofossils of several Trichoptera associated with stony substrate were found exclusively in the older (mostly pre-1900s) Hawes Water sediment. At present, loose sediment and locally abundant filamentous algae in the reedswamp are a threat to the remaining *Chara* species growing in the shallow water.

The decline in Potamogetonaceae and Characeae in Hawes Water can be largely attributed to changes in light limitation, as the steep shelving of the lake leads to large % colonisation

reductions for relatively small changes in potential colonisation depth (see e.g. Vestergaard and Sand-Jensen, 2000). *C. rudis* disappeared from Hawes Water over the course of approximately 20 years, recorded growing at 7 m in 1982, and 4 m in 1999 when it was last recorded, a reduction which corresponds to the loss of approximately 20% of the benthos. Currently, the deepest-growing charophyte is *C. aculeolata*, growing to approximately 2.5 m amongst the Nymphaeaceae and also spilling over steep marl shelves. Although theoretical depth limits would allow for *Chara* growth to 5 m, this is not the case, demonstrating the ease with which angiosperms can outcompete charophytes when light becomes limiting and nutrients are abundant (Ozimek and Kowalczewski, 1984; Hargeby et al., 1994; Middelboe and Markager, 1997; Schwarz and Hawes, 1997; van den Berg et al., 1999). This balance is beautifully demonstrated in Lough Corrib (Ireland) where in the less enriched areas a number of charophyte species grow to depths of 6 m in contrast to more enriched areas dominated by (dense) *Potamogeton pectinatus* and filamentous algae (Krause and King, 1994).

The changes in macrophyte composition indicating deteriorating light condition are in agreement with historical records of changes in the plankton. For example, phytoplankton abundance (as chl *a*) has more than doubled between 1999 (max around 10 $\mu\text{g L}^{-1}$ J. Marshall, unpubl.) and 2009 (max 28 $\mu\text{g L}^{-1}$). Correspondingly, macrofossils of *Daphnia* and *Simocephalus* spp., plankton-feeding cladocerans, increase towards the top of the core. Palaeolimnological pigment analysis shows increases in algal abundance through the 19th and 20th centuries, involving large changes in cyanobacterial communities in the late 19th century and also successional change away from nitrogen-fixers in the earlier half of the 20th century (Chapter 3). Importantly, macrofossil data show that reductions in charophyte fertility (oospore production) seem to have occurred already in the early 1800s, coincident with the first increase in fossil pigment concentrations. Hawes Water has undergone eutrophication-driven change for a considerable time.

In deeper marl lakes, where charophytes have been reported growing to depths exceeding 12 m (Spence, 1982; Pybus et al., 2003), the risk of losing littoral habitats through light limitation is higher than in shallower lakes. Further, the sensitivity of moderately deep lakes to nutrient enrichment can be particularly high owing firstly to the limited shallow littoral (macrophyte) zones and secondly to relatively small water volumes, both of which at extreme ends (very shallow/large, very deep lakes) buffer nutrient enrichment (Genkai-Kato and Car-

penter, 2005). High residence time may increase the sensitivity of moderately deep lakes to enrichment (Moss et al., 1997). In this regard, colonisation depth boundaries are highly applicable as indicators of ecological status for shallow (marl) lakes (mean depth 3 - 15 m).

Similarly to Malham Tarn, based on historical and observational data it is argued here that Hawes Water is in Moderate status, only unequivocally indicated by colonisation depth. Contrary to what was hypothesised, the suggested status is more closely reflected in the status indicated by chl *a* and TP (G, G/M), whereas several macrophyte indices yield High status. The performance of compositional metrics that do not take into account colonisable area and influence of changes in colonisation depth can lead to misclassification, and more “traditional” indices such as TP and chl *a* may in these cases be more reliable unless colonisation depth is directly measured. Further reduction in light penetration due to increasing pelagic productivity is a major threat to the ecological status of Hawes Water.

2.5.3 The ecological status of Cunswick Tarn; identification of a degraded ecosystem

Based on macrophyte colonisation depth, Cunswick Tarn is in Moderate status, whereas chl *a* and TP indicate Poor status (Table 2.10). As with Hawes Water, WFD macrophyte composition indices rank higher, yielding an overall Good status. Through the one-out-all-out rule, however, chlorophyll takes precedence and yields a final classification into Poor status. Palaeolimnological and historical data covering the last few centuries indicate substantial biotic change, and hence a large deviation from reference conditions in line with Poor status, though due to a complete loss of charophytes, Bad may be more appropriate. The ecological consequences of the loss of charophytes from marl lakes is reflected in CSM, which characterises Cunswick Tarn as “destroyed”.

The dramatic change in macrophyte community composition is not reflected by the macrophyte community indices yielding Good status. In this case, the macrophyte community composition table is a very good reference for indicating shortcomings in WFD indices (Table 2.11) (Willby, 2005). In sites with low diversity, it is advisable to supplement macrophyte indices with the simpler “traditional” indices (TP, chl *a*). However, even these indices indicate Poor rather than Bad status. The relatively high colonisation depth in Cunswick Tarn, and the absence of Bad status indicators. compared with the observed ecological degradation, can

in part be explained by particular characteristics of the lake.

Cunswick Tarn is fishless, and therefore supports substantial numbers of large-bodied *Daphnia*. Data also indicate that the population dynamics of *Daphnia* follow a pattern established for meso- eutrophic lakes (Coveney et al., 1977; Lampert et al., 1986; Sommer, 1986; Vanni and Temte, 1990), involving seasonally high limitation of phytoplankton (Figures 2.13, 2.14). This is reflected in the relatively low median chl *a* compared with the mean (44 vs 84 $\mu\text{g L}^{-1}$). Seasonal suppression of phytoplankton, on the one hand leading to the median-mean chl *a* discrepancy, on the other allowing relatively high macrophyte colonisation depth and biomass, is highly likely to explain both the difference between the classifications by chl *a* and TP, and colonisation depth, and the absence of values indicating Bad status. Alternatively, the pattern may be explained by the well-documented variation of ecological status for given concentrations of P (Jones and Sayer, 2003; James et al., 2005; Liboriussen and Jeppesen, 2006), which is also evoked for the alternative stable states hypothesis (e.g. Scheffer et al., 1993; Ibelings et al., 2007).

Historical data are invaluable in assessing the status of Cunswick Tarn by virtue of giving a glimpse of how the lake appeared in the past. For example, nine macrophyte species, including all charophytes, have been lost. The earliest species to disappear were *P. praelongus*, *C. curta* and *C. globularis*, although an exact time point cannot be given owing to the scarcity of records. *C. aculeolata* was last recorded in 1969, *P. natans*, *C. hispida* and *N. opaca* in 1983, and *P. obtusifolius* in 2004. These records indicate that species loss has taken place over a relatively long time frame. Further, *P. australis* was reported spreading to the detriment of *C. mariscus* already in the 1950s. The fossil record supports these observations, revealing firstly a diverse and benthic-dominated community in the early 1900s, secondly an increase in angiosperms with a reciprocal decline in charophytes in the 1930s, and finally increasing abundances of pelagic cladocerans and cyanobacteria since the 1930s, with a decline in mollusc abundance (recording reduced habitat and/or oxygen) over the latter half of the century (Chapter 4). Importantly, the abundant charophyte community in the early 1900s was highly likely an early response to eutrophication and reference conditions for Cunswick Tarn may involve relatively low % cover and potentially lower species richness. LMNI indicates that the early 1900s hydrophyte community in Cunswick Tarn reflected High ecological status, which is supported by the low-impact association of these species based on Table 2.11.

Observational data, in addition to historical data, indicate substantial ecological degradation. The relatively early dieback of macrophytes in comparison with Malham Tarn and Hawes Water, and relatively high and persistent chl *a* throughout the year, indicates stress on macrophytes accumulating over time (Sayer et al., 2010). Haemoglobin-rich cladocerans, and the shallowness of the epilimnion combined with anoxia in the hypolimnion, reveal high respiration rates in the water and sediment, which is an unambiguous indicator of eutrophication in stratifying water bodies (Hutchinson, 1938; Hollander and Smith, 2001; Foley et al., 2012). Finally, although the main inflows to the lake precipitate marl and are clear, no tangible or visible marl precipitation occurs in the lake. In light of the evidence of change in Cunswick Tarn, it is argued here that the lake is in Bad status, which is not portrayed by any of the metrics, and only through CSM assessment. Based on the evidence for this site, the designation of marl lakes with low diversity could benefit from downweighting composition metrics, and giving more weight to simpler indices such as TP, chl *a* and colonisation depth. A CSM-based approach on presence/absence of charophytes could also be considered as part of the rules used to adjust the EQRs of macrophyte metrics. Oxygen profile data may also usefully contribute to ecological status assessments.

2.5.4 A note on marl precipitation and marl lake status

Marl lakes in the UK are mostly shallow, and therefore largely defined by their macrophyte communities, consisting predominantly of *Chara* and *Potamogeton* spp. (Palmer et al., 1992; Duigan et al., 2007). Carbonate precipitation, specifically on charophyte surfaces, is also inherent in this definition (Pentecost, 2009). Therefore, a relationship between *Chara* (and other macrophyte) abundance and carbonate precipitation would be expected in shallow marl lakes of relatively low nutrient status, reflected in an inverse relationship of alkalinity and pH over summer months (although not necessarily in cases where precipitation and weathering patterns lead to maximal alkalinity and pH in winter; Ragneborn-Tough et al. 1999). For example, Kenfig Pool (Wales), with mean and median chl *a* of 9 and 6 $\mu\text{g L}^{-1}$, respectively, and charophytes including *C. aspera*, *C. virgata* and *N. flexilis* agg., shows very clear annual cyclicity in pH and alkalinity (Figure 2.16). A similar patterns of alkalinity exists in Lough Carra (Ireland), with mean chl *a* $< 6 \mu\text{g L}^{-1}$ and eight species of charophytes (King and Champ, 2000), and in Hawes Water, where carbonate precipitation is conspicuous over all plant surfaces and especially on *Chara aculeolata* throughout the summer (Figure 2.5).

Further, in Hawes Water the marl coating in *Chara* is retained through the overwintering period.

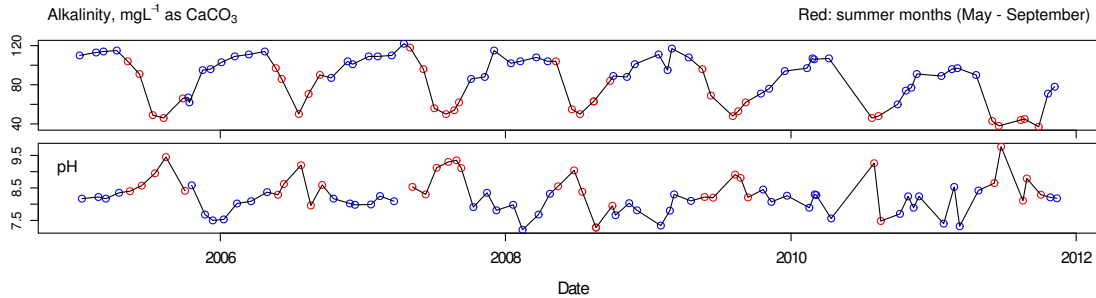


Figure 2.16: Kenfig Pool seasonal alkalinity and pH data collected by the Countryside Council of Wales.

A positive relationship between carbonate precipitation and *Chara* growth has been shown for Malham Tarn in the 1980s (Pentecost, 1984) and to a lesser extent also in the late 1990s (Coletta, 2004), including a clear inverse relationship between pH and alkalinity (Talling and Parker, 2002) (see also Figure 2.18). Against this background, the weak variation in alkalinity, and therefore the weak indication of marl precipitation in Malham Tarn over the study period, was unexpected. Despite having similar TP concentrations and similar ecological status to Hawes Water, marl precipitation was not abundant throughout the sampling period, with only slight *Chara* encrustation observed in 2010 when the pattern of alkalinity and pH also gave indication of precipitation (Figure 2.6). The low variability in alkalinity and pH between the summer and non-summer months in 2009 and 2010 is also evident when compared with long-term records (Figure 2.17) and is difficult to explain. pH in Malham Tarn over the study period was overall higher than in Hawes Water, and would therefore allow precipitation. Secondly, although *Chara* abundance in Malham Tarn has been shown to be cyclical, and related to weather patterns (G. Hinton, unpubl.), with potential consequences for carbonate precipitation, charophyte biomass was high in both 2009 and 2010, strongly suggesting charophyte biomass does not lie behind the low levels of calcite precipitation. Continued monitoring of both pH and alkalinity over all seasons is advisable.

The pattern of carbonate precipitation in Cunswick Tarn is in stark contrast to Hawes Water. In neither sampling year was there a clear relationship between alkalinity and pH to suggest carbonate precipitation, nor was calcite tangible on plant surfaces. While sediment carbon-

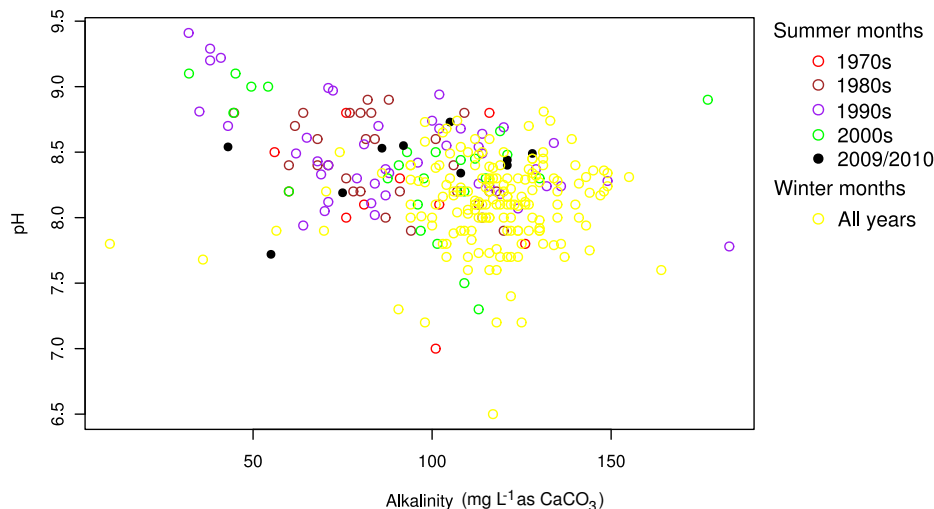


Figure 2.17: Biplot of Malham Tarn pH and alkalinity, data courtesy of the Environment Agency. Summer months are May to September, winter months October to April.

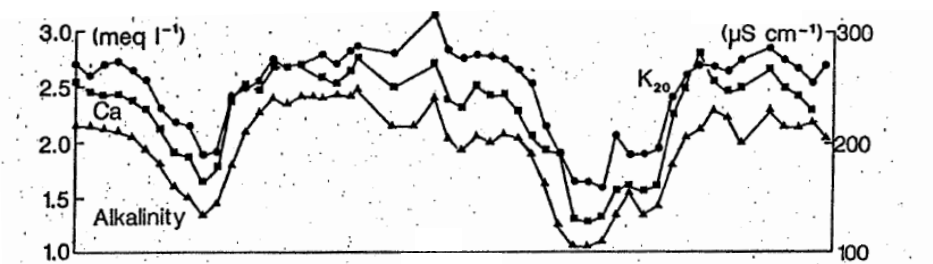


Figure 2.18: Malham Tarn seasonal alkalinity, Ca and conductivity data 1985 - 1987, from Talling and Parker (2002).

ate concentrations evidence some form of calcite precipitation, concentrations are much lower than during the early 1900s when charophytes were a substantial component of the benthos (Chapter 4). This may suggest that the calcite-alkalinity relationship in shallow marl lakes is to an extent driven by benthic (*Chara*) productivity patterns. Megard (1968) showed that, in a number of lakes in Minnesota (USA), calcite saturation was driven by the balance between photosynthesis and respiration. This may in part explain the “disturbed” marling pattern in Cunswick Tarn, as both isotope data (Chapter 5) and O_2 depth profiles suggest Cunswick Tarn has an abundant supply of respired CO_2 and is likely heterotrophic for most of the year, although lakes have been shown to become increasingly autotrophic with eutrophication (Trolle et al., 2012).

2.6 Recommendations for the assessment of marl lake status, and application of WFD metrics

The one-out-all-out approach of both the CSM and WFD is a conservative yet appropriate principle for designating the ecological status of marl lakes. For WFD purposes, this allowed for the control of relatively high macrophyte-based status by chl *a* in both Hawes Water and Cunswick Tarn. However, even in these cases the status seems inflated based on observational and historical data. In Hawes Water, LMNI failed to detect qualitative changes in species composition that have taken place over the course of the 1900s, whereas in Malham Tarn, LMNI specifically depressed the final status classification to Good.

On the basis of the abundance of evidence showing ecological degradation in all three lakes, namely the loss of key macrophyte species and changes in distribution (Cunswick Tarn, Hawes Water, Malham Tarn), decreasing colonisation depth (Cunswick Tarn, Hawes Water), increases in planktonic productivity (Cunswick Tarn, Hawes Water, Malham Tarn), and development of substantial anoxia (Cunswick Tarn), including the potential eutrophication-driven changes in marl precipitation, the following recommendations are made for assessing ecological change in shallow marl lakes. (i) The more conservative TP boundaries suggested by Free et al. (2007) would benefit the assessment of very shallow marl lakes where changes in the benthos precede easily detectable changes in the pelagic zone, (ii) In deeper marl lakes with marked depth zonation, macrophyte colonisation depths should be used in assessments, and chl *a* and WFD TP boundaries appear to be sensitive to overall ecological change compared with macrophyte composition indices.

And (iii), in addition to space-for-time-based indices and classification, palaeolimnological analysis should be considered for WFD purposes, as even relatively low-resolution analysis can give valuable information on key changes within a lake such as successions in macrophyte communities and changes in the balance between pelagic and benthic productivity, and finally (iv) Long-term monitoring data for marl lakes is highly desirable in order to evaluate the relationship between productivity in the benthos and patterns of pH and alkalinity/Ca in the water column.

Table 2.11: Macrophyte community composition along a gradient of ecological status, for most species recorded in the study sites; Table modified from Willby (2005)

Species	High		Good		Moderate		Poor		Bad	
	%	DAFOR	%	DAFOR	%	DAFOR	%	DAFOR	%	DAFOR
<i>Chara</i> spp.	69	A	17	A	4	O				
<i>C. aspera</i> var. <i>aspera</i>	13	A			2	O				
<i>C. aspera</i> var. <i>curta</i>			17	O	2	R				
<i>C. globularis</i> var. <i>globularis</i>	13	F	4	O						
<i>C. globularis</i> var. <i>virgata</i>	19	A	4	F	3	O	6	A		
<i>C. hispida sensu lato</i>	13	A	38	A	4	O	3	R		
<i>Hippuris vulgaris</i>	31	O	46	F	3	O	16	O		
<i>Menyanthes trifoliata</i>	38	F	4	F	4	O	3	O		
<i>Nitella</i> spp.	6	O			2	R				
<i>Nitella opaca</i>	6	O			2	F				
<i>Potamogeton coloratus</i>	13	F	8	O						
<i>P. gramineus</i>	19	O			6	O	6	O		
<i>P. polygonifolius</i>	13	A								
<i>Fontinalis antipyretica</i>	31	O	58	F	45	F	29	O	18	O
<i>Myriophyllum spicatum</i>	25	O	29	F	17	A	29	A	27	O
<i>Nitella flexilis</i> var. <i>flexilis</i>	6	O	8	A	13	F	13	R		
<i>Nymphaea alba</i>	13	O	5	A	6	F	32	F	32	O
<i>Potamogeton berchtoldii</i>	19	F	17	R	16	F	23	O		
<i>P. lucens</i>	6	R	25	A	17	A	35	A	10	O
<i>P. natans</i>	44	O	46	F	53	A	32	O	36	O
<i>P. perfoliatus</i>	31	F	4	F	19	F	19	A	10	R
<i>P. praelongus</i>	25	F	4	O	3	F	10	O	14	R
<i>Utricularia vulgaris sensu lato</i>	13	O	25	O	23	F	6	O	10	F
<i>Callitriche</i> agg.			13	R	17	O	23	O	27	O
<i>Chara globularis sensu lato</i>			4	R	17	F	10	F	27	A
<i>C. vulgaris</i> var. <i>contraria</i>					4	R	3	F	5	F
<i>Elodea canadensis</i>	13	O	54	F	72	A	84	A	68	A
Filamentous algae	25	R	38	O	53	F	59	F	5	F
<i>Lemna minor</i>			46	R	72	O	88	F	68	F
<i>L. trisulca</i>	6	R	5	A	79	A	88	A	64	A
<i>Nuphar lutea</i>	13	R	67	A	83	A	94	A	95	A
<i>Potamogeton crispus</i>	19	R	3	R	16	O	16	A	14	O
<i>P. friesii</i>					2	O	6	A	5	A
<i>P. obtusifolius</i>	13	O	4	R	45	F	55	A	60	A
<i>P. pusillus</i>	6	O	4	R	23	F	23	F	5	O
<i>Zannichellia palustris</i>			4	R	4	O	6	R	5	F

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3 Historical and palaeolimnological evidence for eutrophication-driven declines in macrophyte colonisation depth and diversity in a calcareous lake (Hawes Water, UK)

3.1 Abstract

Hawes Water is a relatively deep mesotrophic calcareous lake that undergoes biologically induced carbonate precipitation during the summer months. Calcification is especially strong on charophyte surfaces, and forms a coating that persists through winter. While the lake is thus currently functioning like a typical marl lake, there have been concerns about increased pelagic productivity and loss of plant colonisation depth despite relatively low mean nutrient concentrations (TP $19.7 \mu\text{gL}^{-1}$, chl *a* $11.8 \mu\text{gL}^{-1}$). To investigate ecological change in the lake with a focus on macrophytes in particular, a palaeolimnological investigation (macrofossils, pigments) was undertaken on two sediment cores collected at 4 and 10 m water depths. Historical records and photographs were used in support. These data indicate an early (1800s) onset of eutrophication involving rapid decreases in charophyte fertility combined with increases in pigment concentration including cyanobacteria. A more pronounced phase follows the 1950s involving declines in species richness and evenness (historical records), turnover in cyanobacterial communities (pigments) and increases in the Nymphaeaceae (macrofossils). Survey data show considerable decreases in macrophyte colonisation depth, related to the extirpation of *Chara rudis*, following the 1980s. This study highlights the sensitivity of (deeper) calcareous lakes to changes in nutrients and light penetration.

3.2 Introduction

Marl lakes are calcite-depositing high-alkalinity lakes widely distributed across the world where local geology supports a continuous supply of carbonates. In deep marl lakes, calcite deposition is brought on mainly by phytoplankton, resulting in fine-grained sediments, in contrast to shallower marl lakes where calcite deposition is associated mainly with benthic macrophytes (Pentecost, 2009). In the UK, most marl lakes are of the shallow type and have therefore been in part defined by their macrophyte communities, falling within category 3140 in the European Union Habitats scheme (EC-DG ENV 2007) as "hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp.", and category I in the British Isles vegetation

scheme as "base-rich lowland lakes, with *Chara* spp., *Myriophyllum spicatum* and a diversity of *Potamogeton* species" (Palmer et al., 1992; Duigan et al., 2007).

The particular macrophytic composition of marl lakes is largely explained by water chemistry. Persistently high pH shifts the balance of carbon species in favour of bicarbonate (Eq. 5), which limits the abundance of macro- and microphytes poorly adapted to assimilate bicarbonate for photosynthesis (Lund, 1961; Spence, 1967; Allen and Spence, 1981; Reynolds, 1998). *Potamogeton*, *Chara* and *M. spicatum* in particular are efficient at assimilating bicarbonate. Importantly, this is also true for the invasive *Elodea canadensis*.



Eutrophication has been identified as the single most important threat to the ecological state of marl lakes in the UK (Pentecost, 2009). In part this is due to the sensitivity of charophytes (key macrophytes of shallower marl lakes) to eutrophication, with declines reported on a national level (Stewart, 2004). The decline of charophyte meadows is in line with established patterns of eutrophication, typified by a transition from lower-growing submerged to taller-growing submerged and floating-leaved species, also involving increases in the abundance of emergent species (Hargeby et al., 1994; Haas, 1999; Davidson et al., 2002; Egertson et al., 2004; Davidson et al., 2005; Ayres et al., 2008; Sand-Jensen et al., 2008; Davidson et al., 2008; Bennion et al., 2009, 2010; Salgado et al., 2010).

However, charophytes seem highly sensitive to eutrophication compared with a number of angiosperms, which may be accounted for by several (covarying) mechanisms. Firstly, decreasing light penetration as a result of increased phytoplankton densities may be the major driver, as angiosperms compete for light more efficiently through rapid shoot growth given sufficient nutrients (for example Ozimek and Kowalczewski, 1984; Hargeby et al., 1994; van den Berg et al., 1999) despite charophytes having lower light compensation points (Middelboe and Markager, 1997). The sensitivity of charophytes to light conditions, independent of changes in nutrient loading, has also been demonstrated (Schwarz and Hawes, 1997). Species-specific depth zonation (Spence et al., 1984; Schwarz et al., 2002) makes charophytes differentially vulnerable to changes in light penetration, and shallow-growing species may persist in lakes

of particularly high nutrient concentrations (Søndergaard et al., 2010).

Secondly, increasing nitrate concentrations may have independent effects on charophytes. Lambert and Davy (2011) found charophyte presence in the UK to be conditional on low nitrate concentrations, and a study by James et al. (2005) revealed strong responses of charophytes in particular to rising nitrate concentrations in a number of UK and Polish lakes. Thirdly, charophytes are disadvantaged by eutrophication-driven changes at the sediment level, such as anoxia, lowered redox state and increases in sulfides (Sederias and Colman, 2009), and structural changes of increasing loose organic matter, impairing rooting and growth (Smith, 2003).

As both charophyte and angiosperm communities change with eutrophication, macrophytic community composition has been promoted as a tool to define the ecological status of lakes (Penning et al., 2008; Willby et al., 2010). However, due to the plasticity of most angiosperms, and the variable relationship of nutrient status with light and sediment conditions, it is somewhat unreliable to rank macrophyte presence/abundance according to nutrient status alone (Søndergaard et al., 2010). Changes within a given lake, either recorded historically or through palaeolimnological analyses, may give more ecologically meaningful information. Specific patterns of loss and increase are evident in such studies, including species associated with marl lakes. Eutrophication-related declines have been shown for *Potamogeton praelongus* (Sand-Jensen et al., 2000; Strand and Weisner, 2001; Egertson et al., 2004; Davidson et al., 2006; Bennion et al., 2009; Salgado et al., 2010), *Potamogeton natans* (Egertson et al., 2004) and *Potamogeton lucens* (Sand-Jensen et al., 2000; Salgado et al., 2010; Sand-Jensen et al., 2008), although changes relating to *Myriophyllum spicatum* are more ambivalent (declines: Salgado et al., 2010; Sayer et al., 2010a) (increases: Strand and Weisner, 2001; Davidson et al., 2002; Egertson et al., 2004; Sand-Jensen et al., 2008; Salgado et al., 2010). These studies show that knowledge of both past and present lake conditions is crucial in developing an understanding of eutrophication responses, and much more useful than reliance on one or the other alone (Sayer et al., 2010b). Palaeolimnology has therefore been flagged as an important tool for defining reference conditions especially for the European Water Framework Directive (WFD) (Bennion and Battarbee, 2007), and for informing conservation and management of lakes (Sayer et al., 2012).

This study therefore applies a combination of limnological and palaeolimnological methods

to Hawes Water, a relatively low-nutrient kettlehole marl lake in northern England. The first aim was to test the null hypothesis that no eutrophication-related ecological change has taken place in the lake, and provided that the null hypothesis was rejected, to investigate the nature of ecological change associated with eutrophication. Mean annual TP ($19.7 \mu\text{gL}^{-1}$) and chl *a* ($11.8 \mu\text{gL}^{-1}$) concentrations place Hawes Water in the Moderate ecological status category for marl lakes (Willby, 2005), implying that the lake is impacted. It was therefore expected that historical and palaeolimnological evidence would reveal eutrophication, primarily through reductions in light penetration (through a decrease in charophyte remains and increases in indicators of pelagic productivity) as well as a decrease in macrophyte diversity, consistent with the hypothesis of marl lake sensitivity to eutrophication.

3.3 Methods

3.3.1 Study site

Hawes Water is a small (5.7 ha) and shallow ($Z_{\text{max}} = 12.2 \text{ m}$, $Z_{\text{mean}} = 4.2 \text{ m}$) mesotrophic kettlehole lake in Silverdale, Lancashire (UK) (Figures 3.1, 3.2 and 3.3). It lies in a shallow basin of Carboniferous limestone in the Gait Barrows Nature Reserve. The catchment is 1.7 km^2 and consists, in addition to the nature reserve, of holiday lets, pastures and cottages. The lake receives water through an artificial inflow connected to Little Hawes Water to the north, and from a series of spring seepages from the lake surrounds as well as in the lake itself. Annual phosphorus (P) loads are estimated at 51.27 kg, and the retention time is approximately 0.32 years (Goldsmith et al., 2003). There is an artificial outflow with associated land drains at the southern end of the lake leading to Hawes Water Moss, the creation of which in the 19th century resulted in lowered water levels. Water levels may later have risen slightly as upkeep of the channels was largely neglected until being cleared out again in the 1960s. Given the steeply shelving bathymetric profile of the lake, areas of shallow water are restricted to the lake margins (Fig. 3.3). Macrophytes become covered by precipitated carbonate during summer, and perennial charophytes retain their carbonate coating through winter.



Figure 3.1: Hawes Water from the southeast, in August 2009.

3.3.2 Limnological monitoring

Contemporary limnological monitoring was carried out for two consecutive years between February 2009 and September 2010, with one winter trip in January 2011. The following parameters were monitored monthly in spring, summer and autumn, and bi-monthly in winter: Soluble reactive phosphorus (SRP), total phosphorus (TP), chlorophyll *a* (chl *a*), nitrate-nitrogen (NO_3^- -N), secchi depth, light penetration, alkalinity, pH, oxygen, conductivity, and temperature. In June and August of both years, semi-quantitative macrophyte surveys were conducted. The frequency and timing of the sampling programme was designed to capture the yearly dynamics of the lake, specifically the interaction between physico-chemical and biological parameters.

The macrophyte community was monitored using standard shore and boat surveys ([JNCC, 2005](#)). At water depths ranging from 25 to >75 cm, 80 m of shoreline was surveyed with the aid of a bathyscope, plants were identified and abundance scores between 1 and 3 were allocated. An overall abundance score of the same scale was given, and the type of substrate was also noted. Boat surveys departed from the centre of the shore section in the direction

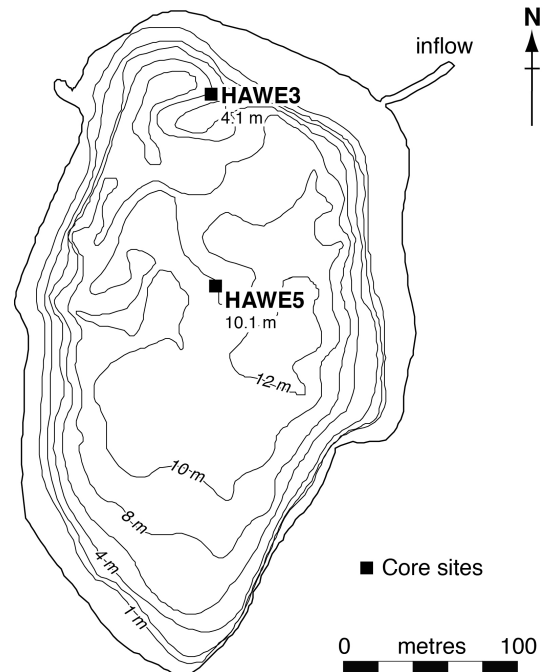
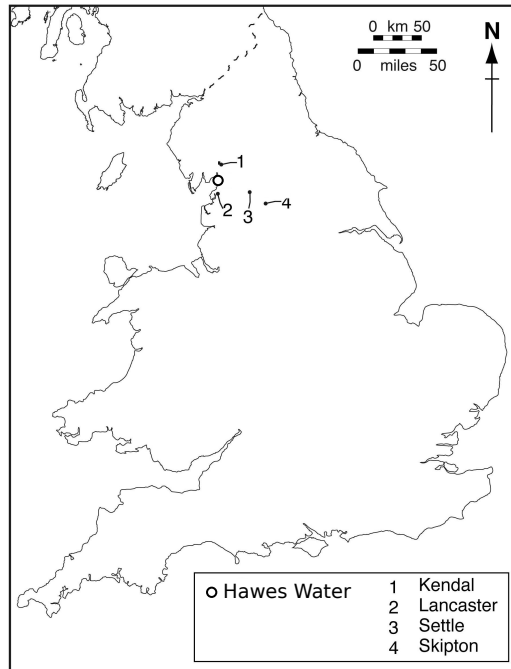


Figure 3.2: A map showing Hawes Water. Figure 3.3: The depth profile and coring locations of Hawes Water.

of the centre of the lake. When possible and necessary, a minimum of 10 points were scored along a depth gradient, with the aim of reaching either the maximum depth, or the depth at which plant growth ceased. The same parameters were scored as for the shore subsections. At each point, with the aid of the bathyscope if necessary, a double-headed rake was thrown in and trawled to evaluate the plant community. The maximum depth of colonisation was tested with several rake throws. Four subsections were selected in total, and additional littoral areas were thoroughly surveyed in 2010 in an attempt to find rare charophyte species. Macrophytes were identified to genus or species level in the field. However, as charophytes are difficult to identify without microscopy, they were sent for identification to Nick Stewart (national recorder for British charophytes).

All chemical and physical monitoring and water collection took place at the deepest point. Surface water was collected in acid-washed 250 ml polyethylene bottles. Chlorophyll *a* samples were collected by filtering a recorded volume of surface water through a 1.2 μm pore size GF/C filter, subsequently stored in aluminium foil and sealed in a plastic bag to prevent photodegradation and contamination. Water passed through the filter was collected for SRP and NO_3^- -N analyses. Samples were subsequently cooled to below 4°C in a portable refrigerator for a maximum of four days. The samples were frozen in the laboratory and analysed as soon

as possible.

Conductivity, oxygen concentration, temperature and pH were measured on-site with Hach® portable IntelliCAL probes on 10 m cables at 50 cm intervals throughout the water column. Since the probes showed different temperatures for a given depth, temperature was consistently read off the pH probe. Light (400-700 nm) was measured with a LI-COR® Biosciences LI-250A light meter on a 9 m cable, and also recorded with 50 cm intervals throughout the water column. If ambient light conditions were unstable, fewer measurements were taken. Alkalinity was measured off-site either immediately after collection or within 24 h. Titration was performed with a Hach® AL-DT test kit to the end-point of pH 4.5 using bromocresol green-methyl red as an indicator.

3.3.3 Water analyses

SRP, NO_3^- -N, TP and chl *a* were determined as soon as possible after collection. Frozen samples were thawed in warm water immediately preceding analysis. Spectrophotometric analysis was performed on a Hach® CAMLAB DR/4000U spectrophotometer with a 1 cm light path. Calibration and blank samples were included in all individual batches for nutrient analysis.

SRP Soluble reactive phosphorus was analysed following the principles of the ascorbic acid method described in Glesceri et al. (1989), with a limit of detection of approximately $1 \mu\text{gL}^{-1}$.

TP Total phosphorus was determined by digesting unfiltered water in a CEM Mars Xpress microwave by the persulfate method described in Johnes and Heathwaite (1992), after which the samples were analysed following the SRP protocol. Care was taken with the addition of phenolphthalein indicator after digestion, since the dye at strong concentrations interfered positively with the spectrophotometric measurements.

NO_3^- -N Nitrate was analysed by conversion to nitrite (assuming naturally occurring nitrite to be negligible) following the method used at the UCL laboratories, according to the principles of the cadmium reduction method described in Glesceri et al. (1989), with a limit of detection of approximately $0.01 \text{ mg NO}_3^- \text{-NL}^{-1}$.

chl *a* Chlorophyll *a* was analysed using the spectrophotometric method described in Glesceri

et al. (1989). At low light, the filter papers were ground in approximately 10 mL acetone by pestle and mortar, followed by centrifugation with a Fisher accuSpin™ 1. The method was simplified by disregarding the acidification step that separates pheophytin *a* from chlorophyll *a*, as the purpose of the analysis was to get an overall quantification of photosynthetic activity in the surface water.

3.3.4 Core collection

Two sediment cores were taken from Hawes Water (Figure 3.3). HAWE3 (71 cm) was taken in October 2009 at a depth of 4.1 m at the northern end of the lake using a wide-bore (internal diameter 140 mm) “Big Ben” piston corer (Patmore et al., *in prep*). The site is a vegetated subsurface mound separated from the lake margins by deeper open water habitat. HAWE5 (31 cm) was taken in January 2011 at the central-northern end of the lake at a water depth of 10 m using a Glew corer (internal diameter 40 mm) (Glew, 1991). Visible downcore changes in sediment colour or structure were noted in the field. The cores were extruded at 1 cm intervals, and sediment samples for pigment analysis (HAWE3) were placed in separate bags and frozen.

3.3.5 Core chronology and lithostratigraphy

Cores HAWE3 and -5 were dated by ^{210}Pb analysis. Freeze-dried sediment samples were analysed for ^{210}Pb , ^{226}Ra , ^{137}Cs and ^{241}Am by direct gamma assay in the Bloomsbury Environmental Isotope Facility (BEIF) at University College London, using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra by the 295 keV and 352 keV gamma rays emitted by its daughter isotope ^{214}Pb following 3 weeks of storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 keV and 59.5 keV (Appleby et al., 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby et al., 1992).

Carbonate and organic carbon content of HAWE3 and -5 were quantified by loss on ignition (LOI) following Dean (1974). The weights after combustion were recorded as percentages

of the dry weight. For % carbonate content, the weight lost was first multiplied by 1.36 to account for the difference in molecular weight of CO_2 and CO_3^{2-} .

3.3.6 Pigments

Pigments were analysed for all HAWE3 sediment intervals at the University of Nottingham laboratories following McGowan et al. (2012). All samples were wrapped in foil to prevent photodegradation, and dried with an Edwards Modulyo 4k freeze-drier prior to analysis, and kept frozen in the dark thereafter. Samples were extracted overnight at 4°C in a mixture of acetone, methanol and water (80 : 15 : 5). Extracts were filtered with a 0.22- μm PTFE filter, dried under N_2 gas and redissolved in a 70 : 25 : 5 mixture of acetone, ion pairing reagent (IPR 0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 mL water) and methanol. Thereafter, analysis was undertaken using an Agilent Technologies 1200 Series high-performance liquid chromatographer (HPLC) fitted with a Thermo Scientific ODS Hypersil reverse phase column (205 x 4.6 mm, 5 μm particle size). An injection volume of 100 μL was used. Analysis followed the method outlined in Chen et al. (2001), with slight modification (Table 3.1). Between 0.1 and 0.4 g of freeze-dried sediment was required to attain sufficient colour for analysis. All samples were analysed in random order and kept in low light conditions at all times. HPLC solvents were degassed before use (Decon[®] FS200b sonicator). All runs included a green standard derived from grass, serving the purpose of showing the retention times of key pigments, and were calibrated against commercial standards to convert peak areas to concentrations.

Pigments were identified by comparison with the absorption characteristics and elution times of standards. Lutein coeluted with zeaxanthin, however owing to the very similar core pattern between lutein/zeaxanthin and canthaxanthin, in contrast to chlorophyte pigments, lutein/zeaxanthin is for simplicity expressed as zeaxanthin. In addition to frequently encountered pigments, a pigment much like pheophytin appeared regularly in the core profile, and was named “cf. pheophytin”. The identified pigments were divided into groups such that total primary productivity is given by the generic pigments chlorophyll *a* and its degradation products as well as β -carotene, cryptophytes by alloxanthin, cyanobacteria by canthaxanthin and zeaxanthin, and chlorophytes by chlorophyll *b* and pheophytin *b* (McGowan et al., 2005). The pigment chlorophyll *a*' refers to the oxidative degradation product divinyl chlorophyll *a*.

Table 3.1: Modifications of the HPLC solvents used by Chen et al. (2001). A: 80% methanol 20% 0.5 mol ammonium acetate, B: 90% acetonitrile 10% deionised water, C: HPLC-grade ethyl acetate

Time (min)	A	B	C	Flow mL min ⁻¹
0	100	0	0	1
4	0	100	0	1
38	0	25	75	1
39	0	25	75	1
43	100	0	0	1
52	100	0	0	1

indicator of grazing (Leavitt and Findlay, 1994)) removed post-suzanne comments The presence of the degradation-prone fucoxanthin throughout the core indicates satisfactory pigment preservation overall. Rapid short-term post-depositional degradation within the top 2 cm was indicated by large changes in fucoxanthin and the degradation profiles of chlorophyll *a*, and these levels were excluded from analysis. Changes in pigment concentrations further downcore are interpreted as independent of preservation effects where ratios between chlorophyll *a* and -b and their degradation products are stable.

3.3.7 Macrofossils

Macrofossils, representing macrophyte, cladoceran, trichopteran, algal, bryozoan and molluscan communities were enumerated every 4 cm for HAW3, and every 6 cm for HAW5.

Approximately 30 cm³ of sediment per sample was used for analysis of both cores. Sample volumes were determined by the displacement of water in a measuring cylinder. Samples were soaked in KOH overnight in order to break down sediment flocs, followed for HAW3 by sieving through meshes of 125 µm and 355 µm, and for HAW5 by sieving through a mesh of 250 µm. Counting was undertaken under a Wild M3Z dissecting microscope. All material of the larger size fractions, and a homogenous subsample of approximately 15% of the small size fraction, was analysed. All macrofossil counts are expressed as numbers per 100 cm³ wet sediment, and remains counted as halves (for example ephippia, statoblasts) are expressed as the minimum number of individuals. Some material was not counted, but given abundance scores between 0 and 3 (high numbers indicate high abundance). This applies to HAW3 *Chara* stem encrustations, and to HAW5 moss, mollusc and Nymphaeaceae remains.

Macrofossils were identified using a reference collection and relevant publications held at UCL

with the exception of *Trichoptera fronto-clypei* that were identified by Malcolm Greenwood at the Department of Geography, Loughborough University, a few moss remains that were identified either as photographs by Graeme Swindles at the Department of Geography, University of Leeds, or as posted samples by Pauline Lang at the Scottish Environment Protection Agency. Where species-level detail was not attainable, remains represent species aggregates. This includes *Daphnia hyalina* agg. ephippia and leaf tips of *Potamogeton pusillus/berchtoldii*. The trichosclereids (leaf cells) of *Nymphaea alba* and *Nuphar lutea* are identical and are grouped as the Nymphaeaceae. Molluscs were mostly only identified to family or genus level. Oospores are very difficult to identify to species level due to substantial morphological variability. Therefore all oospores were aggregated to represent charophytes. However, the different oospore morphotypes found were also counted separately and rough identification was performed using a reference collection based on samples identified by N. Stewart and an identification key (Haas, 1994). “Nude” and calcite-coated oospores were counted separately.

3.3.8 Historical macrophyte records and community composition reconstructions

Species data on past macrophyte presence and distribution (early 1900s to present) were collated from records held by UCL and Natural England, and also by personal communication and journal/image searches on the web. These data, combined with the surveys conducted as part of this study and knowledge of community composition and zonation in comparable marl lakes (Chapter 5, Jupp et al. 1974; Spence et al. 1984; Pentecost 2009; Hilt et al. 2010) or lakes with similar species (Spence, 1967, 1982), and books of charophytes (Moore, 2005) and Potamogetonaceae (Preston, 1995) were used to produce diagrammatic reconstructions of the macrophyte communities and colonisation depths in Hawes Water for the present, the mid-1900s, and the late 1800s/early 1900s.

3.3.9 Data analysis

Due to the possibility of differential preservation of pigments and therefore misleading relative abundances, total pigment abundance was not expressed as summed concentrations. Instead, the downcore data of each pigment were normalised to the maximal abundance of each pigment, giving values between 0 and 1. These were then summed and are referred to as “total abundance”. All data analysis was performed using the statistical software R version

2.12 (R Development Core Team, 2010) with packages analogue (Simpson, 2007; Simpson and Oksanen, 2011), vegan (Oksanen et al., 2011), pcurve (Hastie et al., 2011), and lattice (Sarkar, 2008). Cluster analysis on pigment and macrofossil data was performed on $\ln(x + 1)$ -transformed data avoiding row transformations due to potentially misleading relative abundance data. Ward linkage on Bray-Curtis dissimilarity matrices was used. Similarly, non-metric multidimensional scaling (NMDS) was performed on Bray-Curtis dissimilarities on $\ln(x + 1)$ -transformed data.

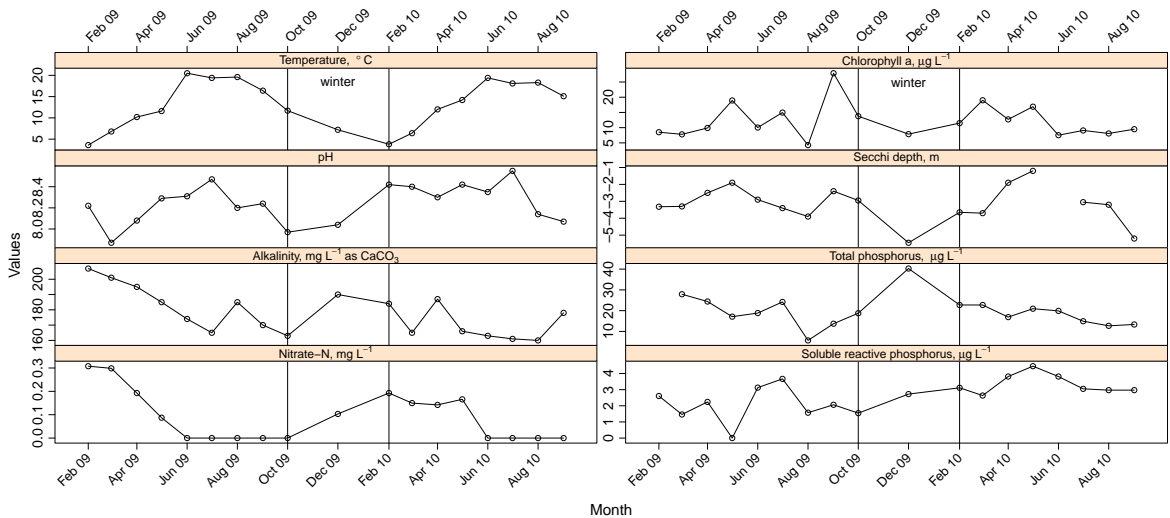
3.4 Results

3.4.1 Contemporary limnological data

Seasonal patterns emerge in the surface water data of 2009 and 2010 (Figure 3.4). Temperature follows the seasons, with minima below 5°C early in the year and maxima around 20°C between June and August. Biological activity follows this seasonality. Peaks in pH corresponding to decreasing alkalinity, reflecting carbonate precipitation, occur during the summer months, coinciding with NO_3^- -N concentrations below the limit of detection. Maximal secchi depth (>5 m) is observed during the winter, and minima approaching only 1 m coincide with peaks in chl *a* concentrations. Chl *a* does not show strong seasonality, with values generally less than 20 μgL^{-1} apart from September 2009 (>25 μgL^{-1}). TP is higher in winter months (max 40 μgL^{-1}) than summer (median annual 18.8 μgL^{-1}), and SRP is constantly at very low levels (<5 μgL^{-1}). Depth profiles of Hawes Water reveal thermal stratification beginning in May/June and ending in September (data not shown) with a thermocline at approximately 5-6 m. Chemical stratification follows the same seasonality, with anoxia developing below 6 m (Figure 3.5). Subsurface oxygen maxima develop in late spring/early summer.

Light penetration (as % of subsurface irradiance [SI]) was variable through the sampling period (Figure 3.6). An SI of 5 was taken as indicative of minimum light requirements for charophytes and taken as a cut-off for maximal colonisation depth (Z_c) (Middelboe and Markager, 1997). The mean Z_c for the sampling period (with depths exceeding 850 cm defaulting to 850) is 515 cm. The “summer” mean (April - September) is 520 cm, and therefore there is no strong seasonality in Z_c . The means for months sampled in both years are largely the same (513 cm (2009) and 538 cm (2010)). These depths are similar to the maximum currently observed colonisation depth of *N. lutea* (550 cm). The theoretical depth limit of charophytes

Figure 3.4: Seasonal surface water data for Hawes Water through years 2009 and 2010. Winter months (October - February) lie between the vertical lines.



is therefore not realised, and angiosperms are occupying this niche.

3.4.2 Current macrophyte communities

Similar macrophyte communities were observed in both the 2009 and 2010 surveys. The margins of Hawes Water were densely vegetated. *Phragmites australis*, *Typha angustifolia* and *Schoenoplectus lacustris* were abundant in the eastern margins, with *Cladium mariscus* particularly abundant along the western margin. *Salix* spp. were occasional in the shallowest areas. Due to the density of reeds along the western edge, very few submerged species inhab-

Figure 3.5: The depth profile of oxygen in Hawes Water through years 2009 and 2010.

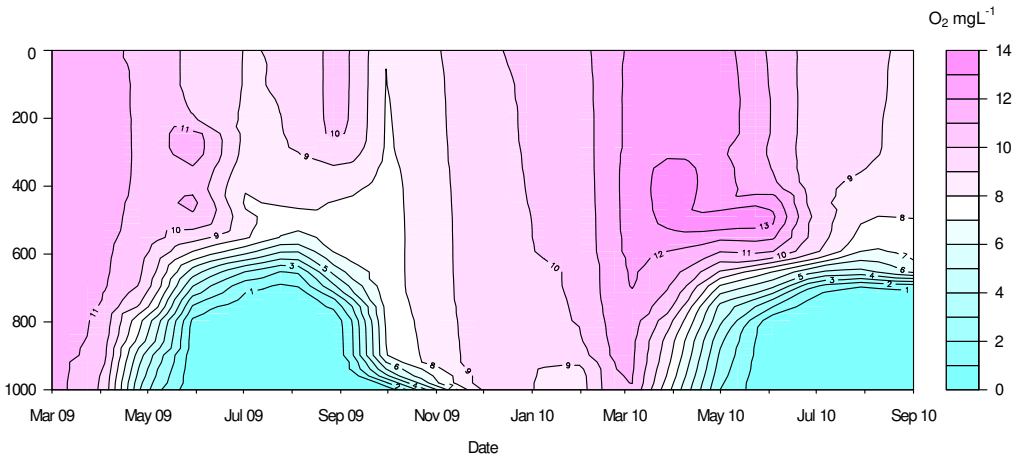
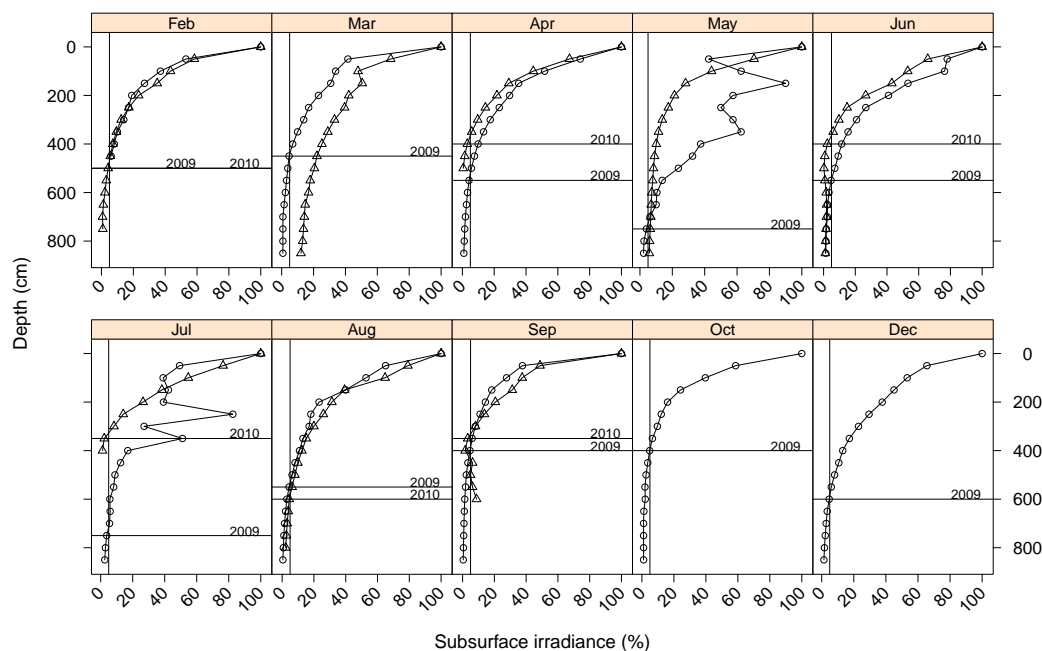


Figure 3.6: Seasonal light penetration data as % subsurface irradiance (SI) for Hawes Water through years 2009 (circles) and 2010 (triangles). Intersecting lines show theoretical depth limits for charophytes assuming a SI of 5 (Middelboe and Markager, 1997). In March and May 2010, SI <5 was below recorded depths.



ited these depths. However, open water patches were found within the margins to the north, east and south, where *Utricularia* sp. (probably *vulgaris*), *Nuphar lutea* and *Potamogeton coloratus* were abundant. In these shallow open patches were also the very rare occurrences of *Chara virgata*, *Chara aspera* and *Chara contraria*. In 2010, relatively abundant filamentous algal growth was noted in these habitats, and sediment was locally very loose. *Fontinalis antipyretica* is locally frequent in the shallower water, and *Lemna minor* as well as *Lemna trisulca* were present locally in the northeast. Reed stems hosted occasional epiphytic algal balls.

The reedswamp became less dense at increasing water depth, zoning into different species communities below approximately 75 cm. At these depths, *Potamogeton coloratus* remained locally abundant, and *N. lutea* became dominant, with submerged mats of *Chara aculeolata* covering marl shelves mainly to depths of approximately 2 m. It was, however, also noted growing to 3.9 m in an area off the regular transects in 2010. Beyond, communities were dominated by *N. lutea*, with maximum colonisation depths ranging from 4 to 5.5 m. Several species existed as patches. *Elodea canadensis*, recorded as rare in 2005, was only found during the 2009 survey in one location to the northeast. *Potamogeton lucens* was recorded to

depths of approximately 4 m in scattered areas along the southwest/south/southeast margins, where occasional patches of *Nymphaea alba* were also found. *Hippuris vulgaris* was found by the boathouse on the west margin, and also as rare in a few places going north. The area surrounding the boathouse also included an unidentified ornamental type of pink-tinted *Nymphaea*. *Myriophyllum spicatum* was restricted to a subsurface mound off the north shore of depths in approximately 4 m, a space shared with *N. lutea*.

3.4.3 Historical macrophyte communities

Macrophyte distribution seems to have been less patchy, and communities more diverse, in the past. Species that have disappeared from the record over the course of the 1900s include *Littorella uniflora* (1911); *Chara vulgaris* var. *papillata* (1915); *Chara globularis*, *Chara hispida* var. *hispida*, *Chara rudis* (1999); *Potamogeton crispus*, *Potamogeton friesii*, *Potamogeton obtusifolius* (1969); and *Potamogeton natans* (1995) (Table 3.2). Due to changes in *Chara* nomenclature and taxonomy, some historical species data are ambiguous. For example, although *C. curta* and *C. aspera* were recorded separately in 1999, these two names can also be synonymous. *C. globularis* may also refer to *C. virgata*, in which case the record from 1915 still holds and the species has not disappeared from the lake. Nonetheless, it is likely that evenness has become reduced in the lake, since *C. aculeolata* and *C. aspera* notwithstanding, charophyte species recorded in 2009 were not found in 2010 and it seems that any findings were chance occurrences, reflecting very rare populations. *C. rudis*, previously recorded to depths of 7 m (1982), later 4 m (1999), is now absent, reflecting decreasing colonisation depths to the point of extinction. The northern mound currently hosting *N. lutea* and *M. spicatum* was, during a coring trip in 1993, described as “mostly *Chara*” and named the *Chara* mound (J. Marshall, field notes).

Hippuris vulgaris was recorded, in addition to the west fringe, at north and south ends of the lake in 1982 (noted as “frequent” (F) on the DAFOR scale), and the north end in 1999, showing that the distribution of the species has diminished. This is also the case with *P. lucens*, and *E. canadensis*, also recorded as F in 1982. *Utricularia vulgaris* agg., on the other hand, has increased in abundance, recorded as “rare” in 1982 and currently growing throughout the lake margins in abundance.

3.4.4 Core chronologies and geochemical characteristics

HAWE3

Core HAWE3 is marly throughout, frequently consisting of a mixture of well preserved macrophyte stem encrustations and more watery sediment. Between the core base and 57 cm, the texture is sandy and of light colour. Above, up to 43 cm, the texture becomes finer and more grey in colour, becoming darker and more brown from 43 cm to 10 cm. The core top is similar in texture, but slightly lighter in colour. The % carbonate and organic matter profiles of HAWE3 fluctuate through the core (Figure 3.7a). Overall, carbonate content is very high, ranging from 48 to 57%. The darker brown sediment noted between 43 and 10 cm is reflected in slightly lower overall core carbonate content. % organic matter shows a pattern inverse to carbonate, and is relatively low, fluctuating between 4 and 13%.

^{210}Pb dates were calculated using the constant rate of supply (CRS) model (Appleby, 2001) (Figure 3.8a). The oldest reliably assignable date is 1947, placed at a core depth of 16.5 cm. While the raw CRS dating model places 1963 at 11 cm, this is slightly shallower than that suggested by the ^{137}Cs and ^{241}Am records, and therefore the final chronologies were adjusted to the 1963 $^{241}\text{Am}/^{137}\text{Cs}$ peak. Also, the CRS-derived 1986 layer is at 6.5 cm, deeper than the 1986 Chernobyl ^{137}Cs peak, which may imply that some of the surface sediments in the core are missing. The ^{210}Pb model indicates that sediment accumulations were relatively stable with an average of c. $0.077 \text{ g cm}^{-2} \text{ yr}^{-1}$ for the last sixty years.

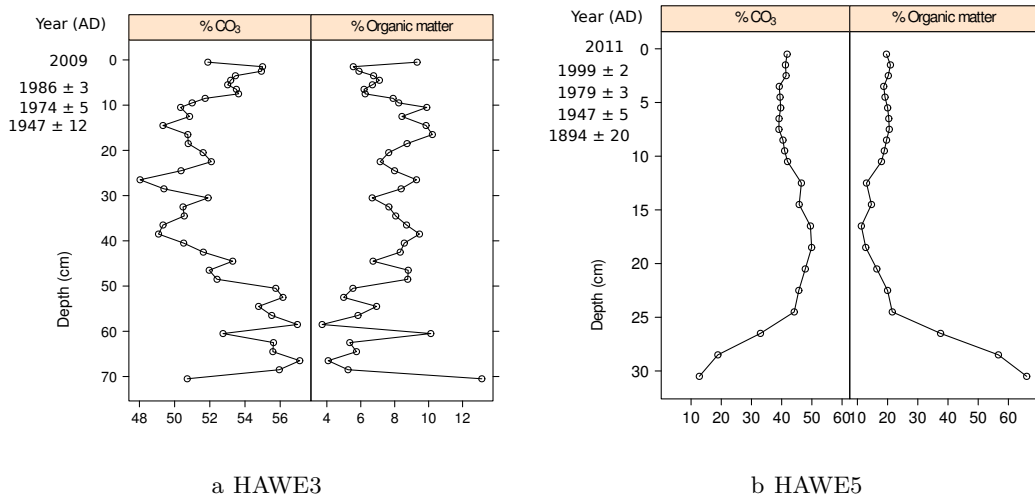


Figure 3.7: Loss-on-ignition data for cores HAWE3 and HAWE5, with ^{210}Pb -derived dates with errors.

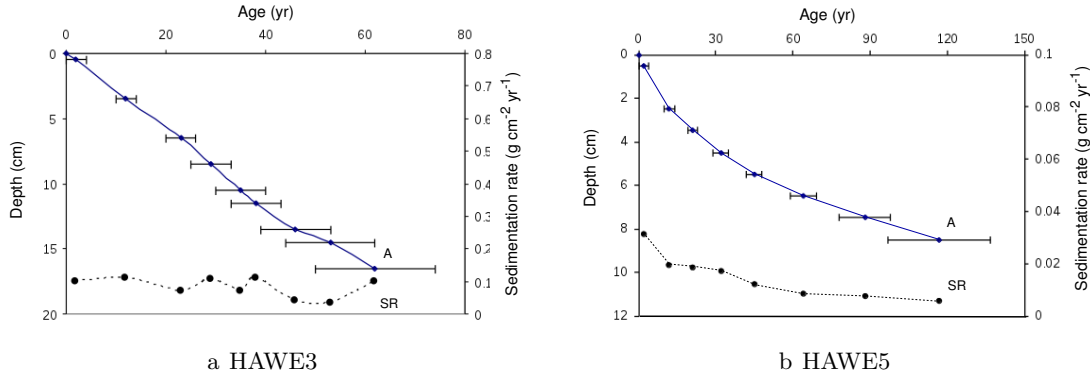


Figure 3.8: CRS-derived age (A; with error margins) and sedimentation rate (SR; dotted line) along the depth profiles of HAWE3 and HAWE5. (Analyst: Handong Yang)

HAWE5

HAWE5, being a deep water core, consists of much finer sediment than HAWE3 and is consequently characterised by lesser carbonate and organic content fluctuations (Figure 3.7b). Between the core base and 24.5 cm, carbonate content increases steadily from 13 to 44%. The high organic content at the core base is reflected by the dark brown/black colour, becoming gradually lighter upcore. At 24.5 cm, carbonate content stabilises and fluctuates only slightly between 39 and 50% around a mean value of 43, showing a pattern of slightly lower concentration above a core depth of 11.5 cm. The small change at this boundary was visible as a shift from grey brown to predominantly brown sediment in the intact core. As for core HAWE3, % organic matter shows a pattern inverse to that of carbonate content, ranging from 66% at the core base to 22% at 24.5 cm, thereafter fluctuating between 11 to 22% around a mean of 18%. The changes in carbonate content also show as marked changes in the colour of suspended sediment of macrofossil samples undergoing KOH treatment (Figure 3.9).

^{210}Pb dates were calculated using the constant rate of supply (CRS) model (Appleby, 2001) (Figure 3.8b). The CRS-derived 1986 layer is at 5.5 cm, in good agreement with the peak in ^{137}Cs . The deepest dated sediment level is 8.5 cm, assigned to 1894, and therefore, in the deeper parts of the lake, sedimentation rates have been relatively low compared with the littoral. The first half of the 20th century is covered by 7.7-6.5 cm, and the latter half by 5.5-2.5 cm, showing a higher sedimentation rate than previously (>0.01 vs <0.01 $\text{g cm}^{-2} \text{yr}^{-1}$). The 21st century is represented by the section from 1.5 cm to the core top, where sedimentation rate is approximately twofold compared with the late 1990s (>0.03 $\text{g cm}^{-2} \text{yr}^{-1}$).

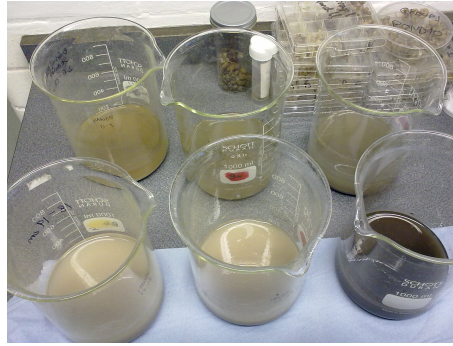


Figure 3.9: From left to right, and top to bottom, HAWE5 sediment from levels 0.5, 6.5, 11.5, 17.5, 23.5, 30.5 cm.

Cross-comparison of core depth ages

The dating profile of HAWE5 (to 1894) is extensive compared with that of HAWE3 (to 1947), which hinders comprehensive cross-comparison (Figures 3.8a, 3.8b). Owing to the fluctuation of sedimentation rates towards the base of the dating record of HAWE3, extrapolation of sediment age further downcore is not reliable. The sediments of both cores are characterised by abrupt changes in the amount of terrestrial wetland material, also mirrored by patterns of *Juncus* seed and *Chara* oospore abundance. Therefore, the cores are approximately and cautiously correlated by these changes in the macrofossil record, which indicates that HAWE3 sediment below 48.5 cm is highly likely to predate the 1800s. Sediment above 48.5 cm may either predate the 1800s or represent the early 1800s.

3.4.5 Stratigraphic clusters and community change

Four major stratigraphic groups are evident in HAWE3 pigment and macrofossil data. Although comparison of the respective cluster boundaries is limited owing to the lower resolution of the macrofossil data, similar clusters are evident for the core base and top (Figure 3.10). Also, for both pigments and macrofossils, core base samples form a separate cluster to all other core levels (Figure 3.10). The base clusters of pigment and macrofossil data are, respectively, base (70.5 cm) to 50.5 cm, and base (68.5 cm) to 48.5 cm.

The difference in pigment (37.5 cm) and macrofossil (28.5 cm) cluster boundaries in the middle section of the core indicates differences in the timing of community change. Further, cluster analysis shows that pigment samples 49.5 - 37.5 cm are more similar to the upper core (from 12.5 to 2.5 cm) than to levels between 36.5 and 13.5 cm. This is reflected by a relatively

large ecological distance between 36.5 - 13.5 cm and all other clusters in ordination space (Figure 3.10). For macrofossils, ecological change is more monotonic through time, with 44.5 - 28.5 cm in one cluster, and 24.5 - 16.5 cm and 12.5 - 0.5 cm in another (Figure 3.10).

Levels demarkating clusters correspond to large community changes (Figure 3.10). The assignment of a separate cluster to the uppermost 12.5 cm in both data sets indicates relatively simultaneous change across taxa from the 1960s to the present day.

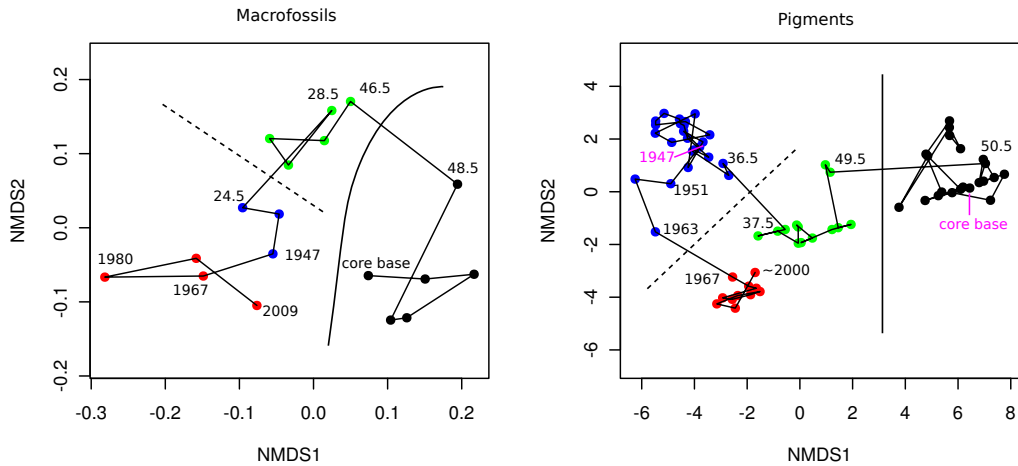


Figure 3.10: NMDS on HAWE3 macrofossil and pigment data. Core levels, and where available, dates as years, are shown between the core base and top where larger community shifts take place. The record of pigments ends approximately in 2000 due to omission of core top levels undergoing rapid post-depositional degradation and therefore containing incomparable concentrations. Colours represent different clusters, solid lines a major cluster division, and dashed lines subordinate cluster divisions.

3.4.6 Pigments

The four pigment zones identified by cluster analysis correspond in time to the pre-1800s, pre-1800s/1800s, 1800s/1900s to 1963, and 1967 - 2000 (Figures 3.11, 3.12). The clusters are differentiated primarily by overall pigment abundance, but also by successional changes through time.

Primary production (chl *a*, degradation products thereof, and β -carotene) is very low in Zone 1 (Figure 3.12), with a few minor peaks in diatoxanthin, chl *a* and fucoxanthin (Figure 3.11). In contrast, there are notable increases in primary production in zone 2, in particular for pigments of siliceous algae (diatoxanthin, fucoxanthin), chlorophytes (chl *b*, pheophytin *b*),

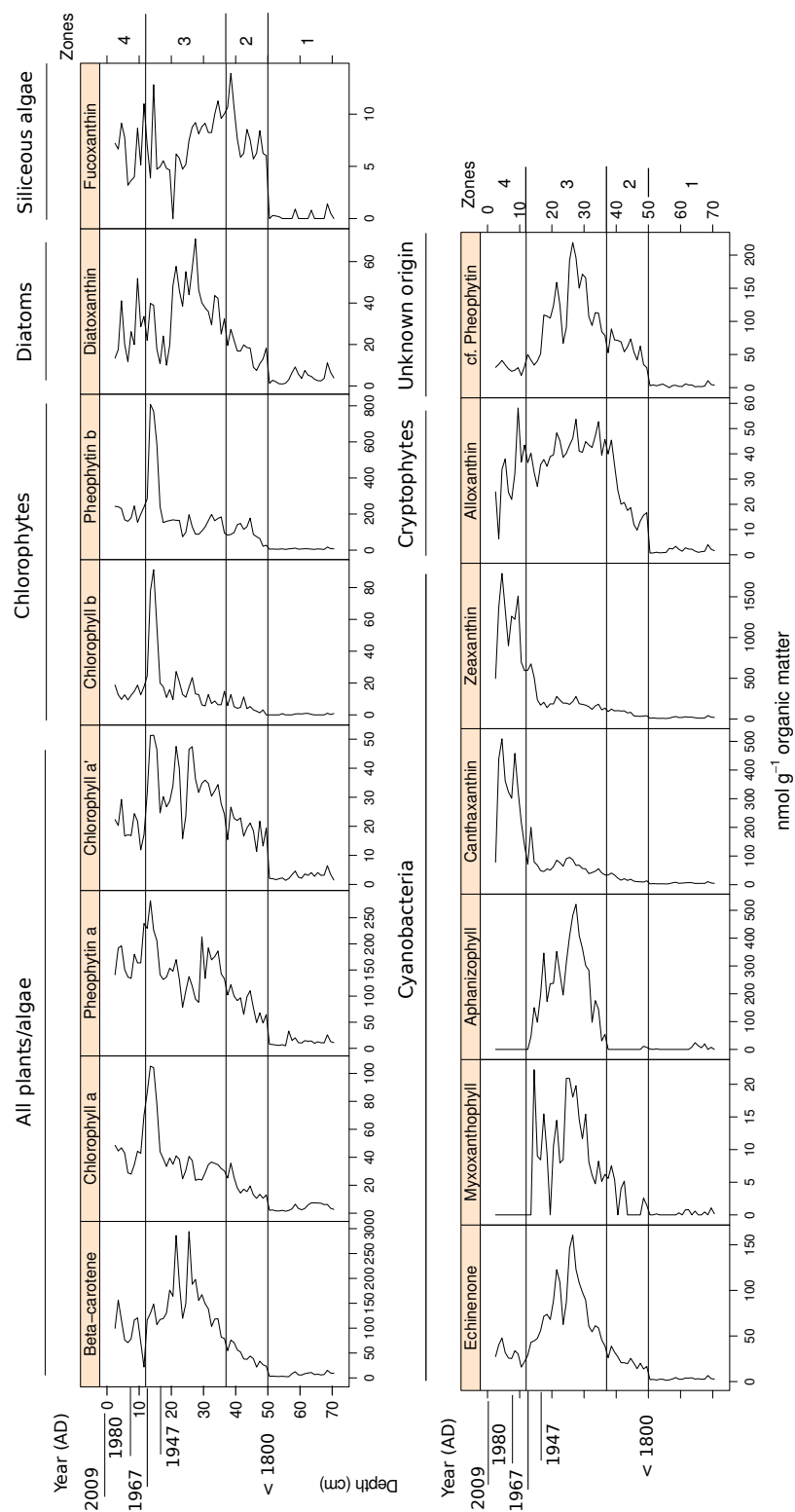


Figure 3.11: Pigment data of HAW-E3 with pigments ordered by group.

and cryptophytes (alloxanthin). Cyanobacterial pigments do not follow the same pattern - increases in myxoxanthophyll, canthaxanthin and zeaxanthin in this core section are very modest, and aphanizophyll is only found occasionally above detectable levels.

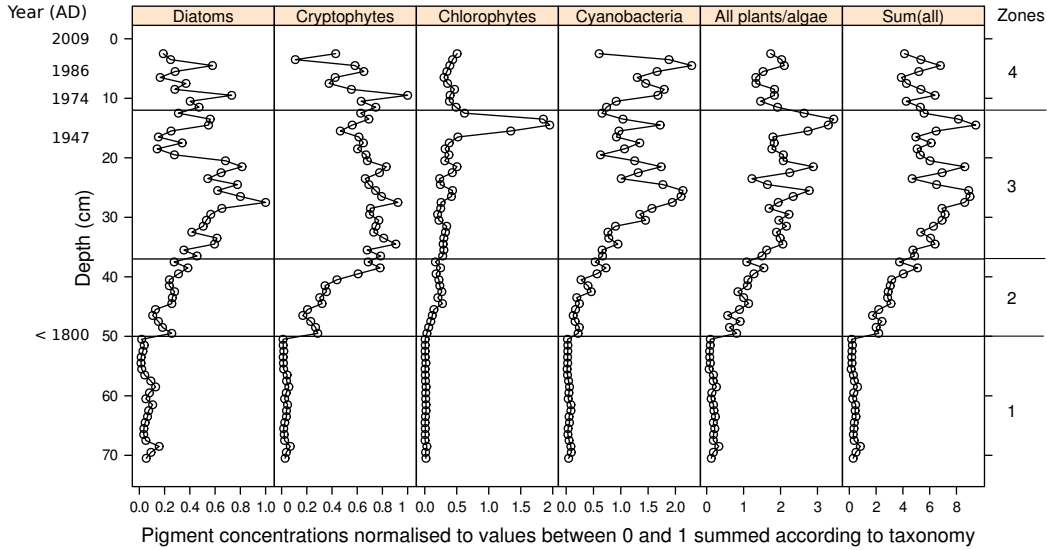


Figure 3.12: HAWE3 pigment data as taxonomic totals, and as total abundance.

In contrast, large changes in cyanobacterial pigments characterise Zone 3 (Figure 3.11). Echinenone, aphanizophyll and myxoxanthophyll increase steeply to 25.5 cm, and display a variable, but decreasing trend, upcore. This pattern is also displayed by β -carotene and diatoxanthin. Concentrations of alloxanthin are relatively stable in this core section, and fucoxanthin is variable with no marked trend of increase or decrease. Primary production increases between 36.5 and 25.5 cm, leveling off thereafter and increasing in variability. The upper end of the core section (16.5 - 13.5 cm; 1940s - 1960s) envelops a distinct peak in the chlorophylls that does not reflect changes in preservation as indicated by stable chl *a*:degradation product values.

The transition to zone 4 (13.5 - 12.5 cm; 1960s/1970s) marks a succession in cyanobacterial pigments (Figure 3.11). Aphanizophyll and myxoxanthophyll decrease to undetectable concentrations, and echinenone decreases to relatively low concentrations, coincident with sharp increases in canthaxanthin and zeaxanthin. Chlorophylls decrease to concentrations similar to those preceding the distinct peak around 14.5 cm, and primary production does not change through this period of compositional change (Figure 3.12). Pigments not showing dramatic

or clear directional changes in the top 12.5 cm include β -carotene, fucoxanthin, diatoxanthin and alloxanthin.

3.4.7 Macrofossils

The four macrofossil zones of HAWE3 correspond in time to the pre-1800s (core base to 48.5 cm), pre-1800s/1800s (44.5 to 28.5 cm), 1800/1900s to 1947 (24.5 to 16.5 cm), and 1967 to 2009 (12.5 cm to the core top) (Figure 3.11). For coherence, data of HAWE5 are divided into the same subsections based on dating, as clusters were not analysed for HAWE5 owing to the low resolution of the data. Through the whole HAWE3 core profile, *Chara* oospores fall mostly into two morphotypes, the majority of which are round, large oospores (*Chara hispida* agg.) and the minority, smaller shouldered oospores (*Chara contraria* agg.), both identifications being in agreement with species recorded historically. A small number of large and oblong oospores were also found, but due to plasticity these may also belong to *Chara hispida* agg. One distinctly pear-shaped and large oospore was found at a depth of 52.5 cm, but no logical identification could be performed as it keyed out as a brackish species.

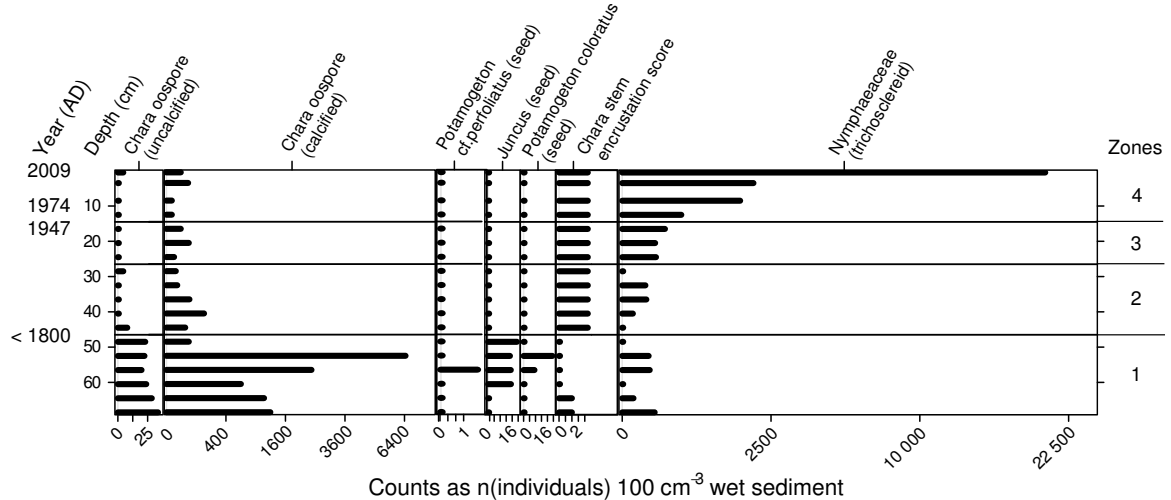


Figure 3.13: Plant macrofossil data of HAWE3.

Zone 1, pre-1800s; core bases to 48.5 cm (HAWE3) and 11.5 cm (HAWE5)

Zone 1 is characterised primarily by an abundance of both moss remains and *Chara* oospores (calcified and nude) (Figure 3.13). These macrofossil samples also stained the sample water, ranging in intensity between amber and black, reflecting influx of peaty material. Although

most moss remains were degraded beyond reliable identification, the most abundant mosses were identifiable as the ombrotrophic moss *Sphagnum austinii*, *Sphagnum* subsection *Acutifolia*, and a hypnoid moss cf. the submerged *Platyhypnidium riparioides*. Post-depositional dissolution of carbonate was evident for this core section. Firstly, mollusc shells were pock-marked, and secondly, rounded indistinct marl clusters were abundant. Thirdly, the abundance of oospores was not mirrored by an abundance of encrusted *Chara* stem remains, and partially dissolved encrustations were noted further upcore at 44.5 cm, indicating absence in the lowermost core levels due to dissolution.

Other plant remains in this core section of HAWE3 include abundant *Juncus* seeds (60.5 - 48.5 cm), a small number of *Potamogeton coloratus* seeds (56.5, 52.5 cm) and one seed of *Potamogeton* cf. *perfoliatus* (56.6 cm) (Figure 3.13). Leaf cells of Nymphaeaceae are relatively low in abundance and display no consistent pattern. Also molluscs (Sphaeriidae, *Lymnaea* cf. *peregra* and *Bithynia* spp.) show no overarching pattern in this section, although a possible decrease in abundance is indicated between 52.5 and 48.5 cm (Figure 3.14). In contrast, round and oblong morphotypes of *Plumatella* statoblasts increase approximately fourfold between these two core levels. No ephippia of pelagic cladocerans were recorded in this core section, and carapaces of the plant-associated chydorid *Pseudochydorus globosus* are relatively low in number.

This core section of HAWE5, correspondingly (and tautologically) also contains high numbers of *Juncus* seeds and moss remains (Figure 3.15). Molluscs and *Chara* oospores are relatively abundant. Nymphaeaceae remains are occasional, and a small number of *Nymphaea alba* seed fragments were found at 24.5 cm. Although not historically recorded in the lake, *P. berchtoldii/pusillus* leaf tips were recorded at 11.5 cm.

Trichopteran head shields are infrequent and low in number in both cores in this section, with species in common including *Limnephilus marmoratus*, *Ecnomus tenellus* and *Mystacides longicornis* (Table 3.3). Further, HAWE 3 contains *Mesophylax impunctatus*, *Mystacides azurae*, *Sericostoma personatum*, and one specimen identified as Limnephilidae only. HAWE5 contains *Oecetis lacustris*, *Athripsodes aterrimus*, *Holocentropus piscicornis*, *Polycentropus irroratus*, one specimen identified as *Tinodes/Lype*, and one as *Limnephilus* sp. It is noteworthy that eight out of the ten species found in HAWE5 occur at 23.5 cm, six exclusively in this level (Table 3.3). *M. impunctatus*, *S. personatum*, *P. flavomaculatus* and *O. lacustris* are

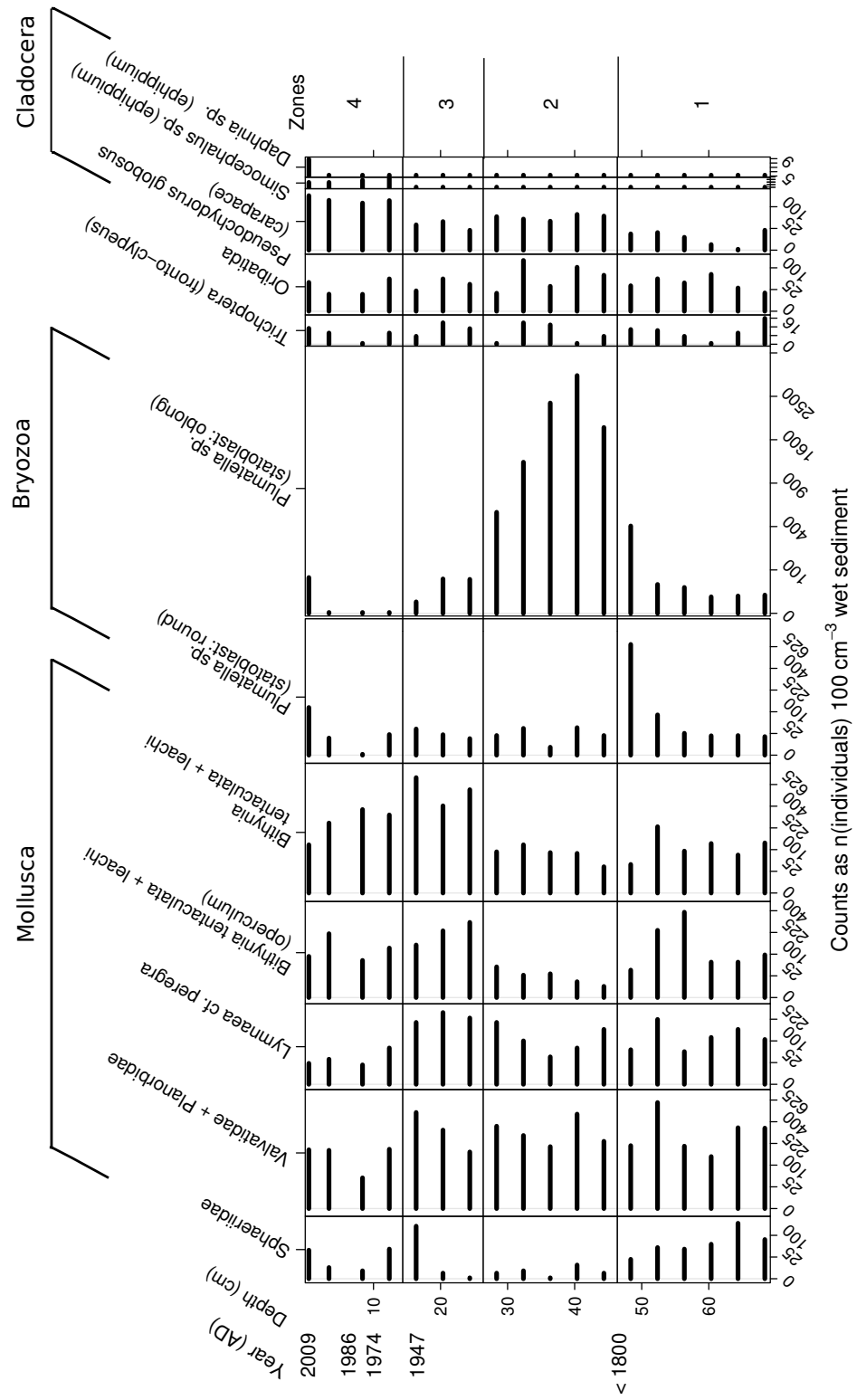


Figure 3.14: Animal macrofossil data of HAWE3.

species associated with stony, exposed substrata. The other species are not exclusively found in one habitat, although all aforementioned species are associated with still, or slow-flowing water.

Zone 2, 18/19th centuries; 44.5 to 28.5 cm (HAWE3); no analysed core levels in HAWE5

The influx of terrestrial matter was reduced during the period covered by this core section in HAWE3 compared with the pre-1800s, indicated by only a slight yellow tint in sample water, lower abundance of moss remains, and no evidence of calcite dissolution in macrofossil material. There are also differences in key macrofossils. Firstly, although encrusted *Chara* stems are abundant throughout this core section, the abundance of oospores (100 cm^{-3}) is markedly lower (100s) compared with the earlier core section (1000s) (Figure 3.13). Secondly, no seeds of Potamogetonaceae or *Juncus* were recorded. In contrast, the abundance of Nymphaeaceae remains are similar to the earlier core section.

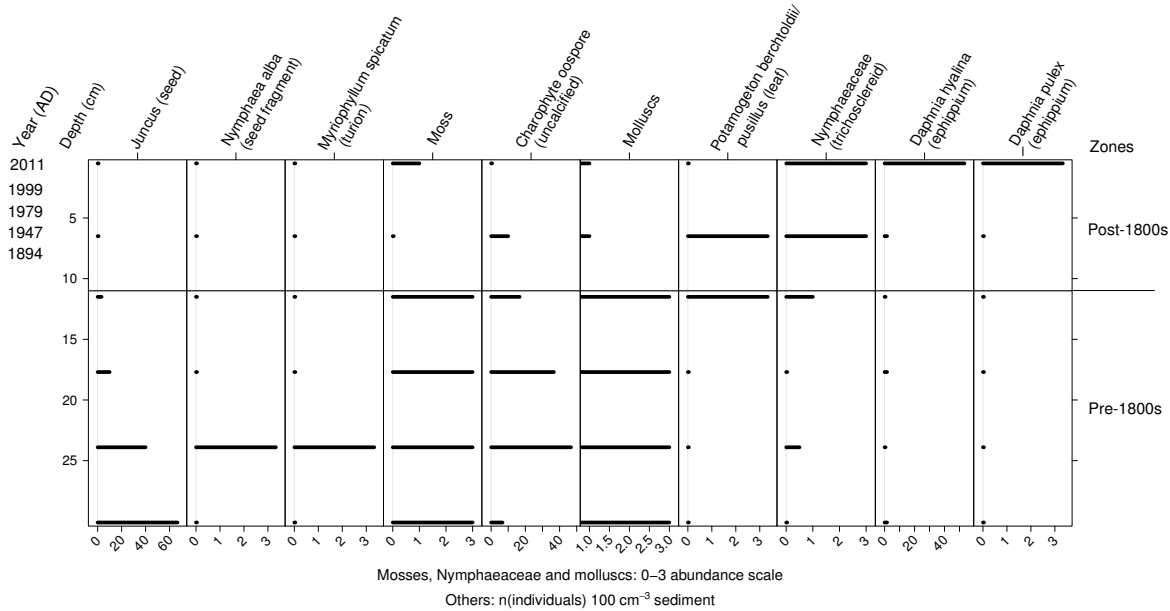


Figure 3.15: Macrofossil data of HAWE5.

Among the mollusc remains, those of *Bithynia* spp. and the Sphaeriidae are lower than in the previous section, and the remaining taxa do not display changes (Figure 3.14). Between 48.5 and 40.5 cm, there is a fourfold increase in the abundance of oblong *Plumatella* statoblasts, followed upcore to 28.5 cm by an equally dramatic decrease. Remains of *P. globosus* are more abundant than in the previous core section, and no pelagic cladoceran taxa were recorded.

Trichopteran head shields in this core section are largely absent ($n = 3$) and those found were towards the base of the section, identified as *M. longicornis*, *Athripsodes aterrimus* and *P. irroratus* (Table 3.3). Only *P. irroratus* is associated with stony substrata, the other showing broad habitat preference including sand, mud and vegetation.

Zone 3, 19/20th centuries to 1947; 24.5 - 16.5 (1947; HAWE3); sample 6.5 cm (HAWE5)

The section transition (28.5 - 24.5 cm) in HAWE3 marks a small increase in Nymphaeaceae remains, a relatively large increase in *Bithynia* spp., and a fourfold decrease in the abundance of oblong *Plumatella* statoblasts (Figures 3.13, 3.14). Further upcore within this section, the abundances of the aforementioned taxa are relatively stable. This core section contains two Trichoptera species, the previously recorded *P. flavomaculatus* (16.5 cm) and the first records of *Cyrrnus flavidus* in the core (20.5, 16.5 cm) (Table 3.3). *C. flavidus* is a species found in a variety of habitats, especially among macrophytes, in still water. In HAWE5, Nymphaeaceae and *P. pusillus/berchtoldii* remains are abundant, however no Trichoptera remains were found (Figure 3.15).

Zone 4, 1967 - 2009; 12.5 - core top (HAWE3) + 2011 (core top: HAWE5)

Zone 4 in HAWE3 shows little change from previous core sections in terms of plant macrofossils. *Chara* stems remain high in abundance, oospores remain low in abundance, and no seeds of Potamogetonaceae were recorded (Figure 3.13). However, there is a large and monotonic increase in Nymphaeaceae remains to the core top, the uppermost sample containing 10 times more trichosclereids than the previous level (3.5 cm). Molluscs show a slight decrease in abundance towards the core top, *Lymnaea* cf. *peregra* and the Valvatidae + Planorbidae in particular (Figure 3.14). Both *Plumatella* statoblast morphotypes are recorded only occasionally. Cladocerans (*Simocephalus* sp., *P. globosus*) increase in abundance between 16.5 and 12.5 cm, then display little variability to the core top. The uppermost sample contains the first core record of *Daphnia* spp. The high abundance of Nymphaeaceae remains, and the appearance of *Daphnia* spp., in the most recent sediment is faithfully reflected in the 2011 sample from HAWE5, where *Daphnia* ehippia are particularly abundant (Figure 3.15). The only Trichoptera species recorded in this core section is *C. flavidus* (Table 3.3).

Table 3.2: Historical records of macrophytes in Hawes Water, and all species recorded 2009/2010.

Date	Flora	Source
2009 & -10	<i>Chara aculeolata</i> , <i>Chara aspera</i> , <i>Chara contraria</i> , <i>Chara virgata</i> , <i>Fontinalis antipyretica</i> , <i>Cladium mariscus</i> , <i>Elodea canadensis</i> , <i>Hippuris vulgaris</i> , <i>Lemna minor</i> , <i>Lemna trisulca</i> , <i>Myriophyllum spicatum</i> , <i>Nuphar lutea</i> , <i>Nymphaea alba</i> , <i>Phragmites australis</i> , <i>Potamogeton coloratus</i> , <i>Potamogeton lucens</i> , <i>Schoenoplectus lacustris</i> , <i>Typha angustifolia</i> , <i>Utricularia vulgaris</i> agg.	Surveys by author
1999	<i>C. hispida</i> var. <i>hispida</i> (syn. <i>C. aculeolata</i>), <i>C. hispida</i> var. <i>major</i> ; <i>Chara rudis</i> to 4 m depth; <i>P. lucens</i>	Survey by C. Newbold
1995	<i>C. aculeolata</i> , <i>C. aspera</i> , <i>Chara curta</i> , <i>C. hispida</i> , <i>C. rudis</i> , <i>P. lucens</i> , <i>Potamogeton natans</i>	1996 Natural England report for Cumbria County Council and ARC
1993	<i>Chara</i> sp. recorded at HAWE3 coring site at depths around 4 m	Field notes by J. Marshall
1982	Encrusted <i>C. aspera</i> abundant, <i>C. rudis</i> to 7 m depth; <i>E. canadensis</i> , <i>P. lucens</i> ; “Water was very brown in colour and visibility was poor.” - M. Wade	Diver survey by C. Newbold et al.
1969	<i>C. aculeolata</i> , <i>C. aspera</i> , <i>C. mariscus</i> , <i>N. lutea</i> , <i>N. alba</i> , <i>P. australis</i> , <i>Potamogeton crispus</i> , <i>Potamogeton friesii</i> , <i>P. lucens</i> , <i>Potamogeton obtusifolius</i>	School trip; J. Birks
1915	<i>C. aculeolata</i> , <i>Chara fragilis</i> subsp. <i>delicula</i> (possibly syn. <i>C. globularis</i>), <i>C. rudis</i> , <i>C. vulgaris</i> var. <i>papillata</i> , <i>P. friesii</i> , <i>P. lucens</i> , <i>P. obtusifolius</i> , <i>H. vulgaris</i> , <i>N. lutea</i> , <i>N. alba</i> ; “The Characeae are in considerable quantity and much encrusted”	W. H. Pearsall, in Druce (1916)
1911	A record of <i>Littorella uniflora</i>	Druce (1911)

Table 3.3: N(Trichoptera) 30 cm⁻³ (wet sediment) found in HAWE3 and HAWE5.

HAWE3	Depth (cm)	Mystacides azurae	Sericostoma person- atum	Mesophylax impunc- tatus	Ecnomus tenellus	Limnephilus mar- moratus	Limne- philidae indet.	Mystacides longicor- nis	Athripsodes aterrimus	Poly- centropus irroratus	Poly- centropus flavomacolatus	Cyrnus flavidus
	0.5										4	
	3.5										1	
	8.5											
	12.5										1	
	16.5									1	1	
	2.5										1	
	24.5										1	
	28.5											
	32.5											
	36.5							1				
	4.5											
	44.5								1			
	48.5								1			
	52.5				1			1				
	56.5					1	1					
	6.5											
	64.5			1								
	68.5	1	1		1			2				
HAWE5	Depth (cm)	Tinodes/ Lype- type	Limnephilus mar- moratus	Agrypnia pagetana	Poly- centropus irroratus	Athripsodes aterrimus	Limne- philus indet.	Oecetis lacustris	Ecnomus tenellus	Mystacides longicor- nis	Holocentropus piscicor- nis	
	0.5											
	6.5											
	11.5									1	1	
	17.5								1			
	23.5	2	1	1	1	1	1	1	1			
	30.5	1										

3.5 Discussion

3.5.1 Spatial representation of Hawes Water by cores HAWE3 and HAWE5

Several cores are ideal for reconstructions at a whole lake scale in order to control for the patchiness of macrophytes in lakes and the generally low dispersal of macrophyte remains (Zhao et al., 2006). As HAWE3 was taken at a subsurface mound of shallower water detached from the lake margins, it is highly likely that macrofossils are mainly local. This is supported by the low number of macrophyte species in the fossil record, while *Chara* remains were numerous in agreement with historical records of presence at the coring site. The seeds of *Potamogeton coloratus*, recorded in samples 52.5 and 56.5 cm, may reflect particularly high abundance of the species in the shallower margins rather than at the coring site, as the seeds can float and this species typically grows in shallower, calm water as found in Hawes Water at present.

The trichosclereids of the Nymphaeaceae are likely to be relatively widely dispersed after senescence of the parent leaf owing to their very small size and star-like shape. However, because parent leaf decay is a prerequisite, high abundances of trichosclereids are likely only local to the beds. This is supported by the core data. In HAWE3, trichosclereids are extremely abundant at the core top in agreement with the present dominance of *Nuphar lutea* on the coring site, yet they are also present in low numbers in HAWE5, collected at 10.1 m water depth closer to the centre of the lake and far beyond the currently recorded maximal colonisation depth of Nymphaeaceae in Hawes Water.

Being a more centrally located core, and given the pronounced slopes of Hawes Water promoting sediment focusing, HAWE5 would be expected to contain a more integrated lake signal, albeit with lower abundance of poorly dispersed remains. This is supported by the presence in the core of not only trichosclereids but also *Potamogeton* leaf tips, which are light and easily transported (Davidson et al., 2005). The presence of the remains of several trichopteran species associated with rocky substrates also suggests a more whole-lake signal.

In contrast to the macrofossils in HAWE3, the pigment data may reflect not only local trends of macrophyte abundance and attached microphytes, but also pelagic microphytes due to wind- and wave-driven influx from the surrounding areas of open water. Therefore no particular assumptions were made regarding the exact source of pigments, however the high

concentrations of cyanobacterial pigments especially further upcore would suggest increasing pelagic sources.

3.5.2 Stages of eutrophication in Hawes Water

The key stages of eutrophication in Hawes Water are the early stages of relatively low impact (pre-1800s/1800s), the acceleration of eutrophication after the mid-1950s, and the development towards the current status of the lake since approximately the 1980s. To better visualise the associated changes in the macrophyte community, the reconstructions of the likely distribution of macrophytes through these stages (see Methods) are shown in Figure 3.16. These diagrams portray the gradual loss of habitat both from the deeper zones and the littoral through time, combined with the recorded reduction in species diversity.

3.5.3 Early signs of eutrophication in Hawes Water

In the pre-1800s, encompassed by zone 1, Hawes Water received large influxes of acidic wetland matter as suggested by the abundance in the macrofossils of mosses (including the ombrotrophic *Sphagnum austinii*), the dark, peaty colour of the sample water, and the dissolution of carbonate remains. It is probable that this corresponds to historical land drainage and therefore erosion of surrounding wetlands, with most allochthonous loading coinciding with core levels between the base and 48.5 cm (pre-1800s) and decreasing upcore until becoming negligible above 24.5 cm (1800s/1900s). The influx seemingly did not have a monumental effect on the biogenic carbonate deposition in the littoral zone as indicated by high core carbonate content including abundant encrusted oospores. However, post-depositional dissolution is highly likely to account for the scarcity of encrusted charophyte stem remains, as in contrast to upper core levels with abundant remains and little evidence of dissolution, the lower levels contain abundant nondescript marl clusters which are likely to be reworked and degraded stem encrustations.

The pattern of increasing oospore numbers upcore within the zone of high terrestrial influx is potentially a response to increasing nutrient supply, a pattern which has been recorded in a similar marl lake, Cunswick Tarn, in the Lake District (Chapter 4). It is plausible that drainage resulted in increased nutrient loading and early eutrophication. With reductions in water table levels, wetland soils become more oxygenated, which enhances decomposition and

Hawes Water

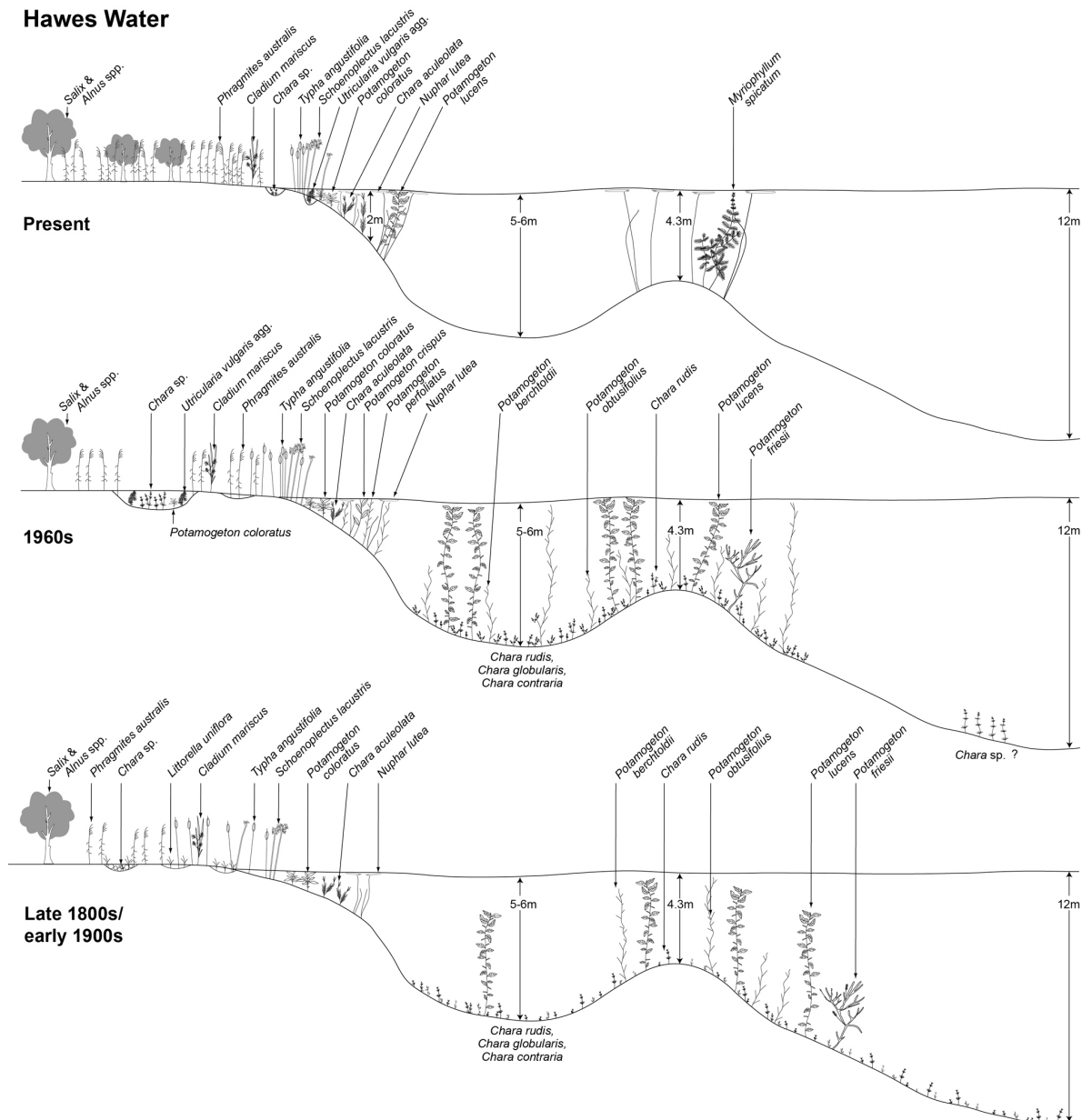


Figure 3.16: Cross sections of macrophyte cover, showing the currently dominant community, and historical communities based on available historical data.

results in increased losses of nutrients into drainage ditches (Snyder and Morace, 1997).

The Sphaeriidae were particularly abundant in zone 1, and declined thereafter. The pattern is difficult to explain owing to the very broad ecological niches of freshwater molluscs (Dillon, 2000). While the Sphaeriidae are generally associated with fine-grained sediments as burrowing filter-feeders (Dillon, 2000), they are extremely abundant on *Chara* in Malham Tarn (pers. obs.). The similar abundance distribution of oospores and the Sphaeriidae at the core base may therefore be a reflection of this association. Increases in pigment concen-

trations indicate increasing productivity at 50.5-48.5 cm (Sanger, 1988; Leavitt, 1993; Leavitt and Findlay, 1994; McGowan et al., 2005; Mikomägi and Punning, 2007), corresponding to a dramatic decrease in oospore numbers, and a slight decrease in the Sphaeriidae. Rapid decreases of oospores coinciding with increases in pigment concentrations have also been described in Cunswick Tarn (Chapter 4), possibly indicating a pattern common to marl lakes undergoing eutrophication.

Despite the reduction in oospore numbers, marl-encrusted charophyte stems were abundant throughout the core, and charophytes were recorded growing at the coring location in 1993, suggesting charophytes did not disappear from the site at this point in time. Therefore, oospore abundance in HAW3 is not linearly related to charophyte abundance. The *Chara hispida* agg. are perennial charophytes and can therefore develop very dense stands over time, with spring growth occurring from starch deposits from senescing nodules further down the stem (N. Stewart, pers. comm.). Seed production in perennial plants is generally related to favourable environmental conditions (Fenner and Thompson, 2005), and it is therefore probable that reduced light conditions with increasing phytoplankton/epiphytes, as evidenced by increasing pigment concentrations, made the habitat more hostile and oospore production diminished in favour of vegetative growth. In support, light-dependent oospore maturation has been shown for various charophyte species (Stross, 1979; Bonis and Grillas, 2002). Stross (1979) also discussed potential photomorphogenetic “switches” relating to light changes induced by the interception of certain wavelengths by phytoplankton pigments. The abrupt oospore decline (1000+ to <100 100 cm⁻³) at 48.5 cm may indicate the operation of such a switch and marks a very early change in the balance of lake productivity from benthic clear-water *Chara* cf. *hispida* agg./*Chara* cf. *contraria* agg. meadows to higher production rates of microalgae.

The decrease in oospores corresponds to a substantial increase in *Plumatella* and decreased total mollusc abundance. Whilst this pattern is impossible to explain ecologically, especially as bryozoans are notorious for undergoing boom-bust cycles (Wood and Okamura, 2005), the continuing presence of molluscs combined with the abundance of *Plumatella*, suggests that detrital, epiphytic and planktonic food sources continued to be abundant. As molluscs and bryozoans are abundant on macrophytes, this also offers support to continuous presence of macrophytes on site, despite reductions in charophyte fertility. Reductions in oospore pro-

duction have been connected with reduced charophyte biomass (Bonis and Grillas (2002), J. Harris, pers. comm.), and historical records of relatively deep-growing *Potamogeton* spp. open up the possibility of a eutrophication-related submerged charophyte - tall-growing angiosperm succession (e.g. Hargeby et al., 1994; Davidson et al., 2005; Ayres et al., 2008). Regrettably, Potamogetonaceae are often underrepresented by seed remains (Zhao et al., 2006) and it is thus impossible to assess this scenario based on the macrofossil record in Hawes Water. However, the relatively low abundance of Nymphaeaceae remains indicates that these early stages of change did not involve an increase in floating-leaved species.

In the pigment data, a continued rise in productivity is evidenced by steady increases in most algal groups from 50.5 cm to approximately 26 cm (Figure 3.12), including increases in filamentous and N₂-fixing cyanobacteria that peak at 25.5 cm. While there is no universal simple pattern of algal succession related to eutrophication (Reynolds, 1998), increases in cyanobacteria, also specifically filamentous forms, are commonly recorded in eutrophying lakes (Sanger, 1988; Leavitt and Findlay, 1994). However, the presence of aphanizophyll, suggesting presence of *Aphanizomenon*, indicates eutrophication was not severe at this time as the species is commonly associated with (relatively) moderate enrichment (Steinberg and Hartmann, 1988; Carvalho et al., 1995; Körner, 2001). The peak in cyanobacteria is broadly coincident with a shift in the macrofossils as shown by cluster analysis. These changes involve a decrease in *Plumatella*, an increase in Nymphaeaceae, and increases within the Mollusca (*Bithynia* in particular). Increases in *Bithynia* may be a reflection of increased suspended as opposed to attached food, as the genus is capable of switching between grazing and suspension-feeding depending on food availability, and may outcompete obligate grazers when pelagic productivity increases (Brendelberger and Jürgens, 1993). Increases in food availability, particularly in the pelagic zone, is supported by the increase in the floating-leaved Nymphaeaceae, usually a response to deteriorating light conditions (Davidson et al., 2002; Egertson et al., 2004; Davidson et al., 2005; Sand-Jensen et al., 2008; Davidson et al., 2008; Bennion et al., 2009, 2010; Salgado et al., 2010).

Despite relatively substantial changes in algal composition and, highly likely, abundance, Hawes Water continued to be in a clear-water, macrophyte-dominated state (Figure 3.16). This is evident from historical records of the site from the late 1800s and early 1900s, encompassing the sediment levels showing increasing cyanobacteria, *Bithynia* and Nymphaeaceae.

For example, the deep-growing *Chara rudis* and *C. globularis*, recorded in 1915, evidence high light penetration and viable charophyte populations at depth despite decreases in charophyte fertility at the coring site already in the previous century. An early photograph of Hawes Water (probably from the late 19th/early 20th century), gives an idea of what the site looked like when benthic communities were dominant (Figure 3.17). The lake margins are remarkably open, with abundant shallow water pools in the reed zone providing a diverse habitat for shallow water submerged macrophytes (Figure 3.17). In agreement, *Littorella uniflora*, a species associated with shallow, open areas, was recorded in 1911 (Druce, 1911), implying limited reed development compared to the present day. This interpretation is also supported by the prevalence, in both sediment cores, of trichopteran species associated with stony substrate, particularly in the pre-1800 era but also into the 1800s. It is likely that siltation of the lake margins slowly led to more reed development (Mäemets and Freiberg, 2004; Liira et al., 2010) and consequently to the extinction of *L. uniflora* from the site.

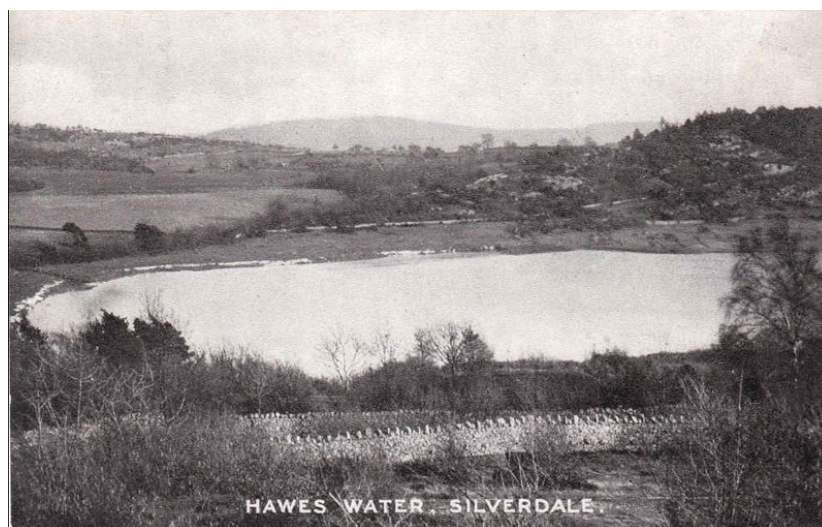


Figure 3.17: Hawes Water in the late 19th/early 20th century. The photo is taken at the east-southeast end of the lake. available at <http://www.historic-images.co.uk/lancashire/silverdale>

3.5.4 Eutrophication-driven changes since the 1950s

The latter half of the 20th century, and the beginning of the 21st century, encompass the most rapid and dramatic change in the macrophyte communities of Hawes Water. This is in contrast to earlier times when the more subtle changes would have been unlikely to be apparent to someone standing at the lake shore. These changes also show acceleration with time, suggesting the resilience of benthic communities are becoming compromised and turbidity is

likely to increase in the near future (see e.g. Scheffer et al., 2009). Reports of more frequent occurrences of “green” water over the last decade (pers. comm.) support recent and rapid changes in phytoplankton abundance.

Around the 1950s (13-14 cm; HAWE3), chlorophytes and pigments indicative of overall productivity displayed a slightly anomalous spike (Figure 3.11), unlikely to be an artefact of changes in preservation as indicated by stable degradation profiles of chlorophylls. Historical aerial photographs give no indication of land use changes at this time, and the pattern is difficult to explain. This spike is nested between a decrease in echinenone, aphanizophyll and myxoxanthophyll, and an increase in canthaxanthin and zeaxanthin, indicating a sequence of substantial shifts in microphytic communities. The cyanobacterial pigment association suggests specifically a decrease in N_2 -fixers and myxoxanthophyll-producing colonial cyanobacteria to species producing canthaxanthin (colonial cyanobacteria) and zeaxanthin. Zeaxanthin is generally not produced by Nostocaceae (heterocystous cyanobacteria including *Aphanizomenon*) (Hertzberg and Liaaen-Jensen, 1971; Steenbergen et al., 1994), a further indication that the abundance of N_2 -fixers diminished following the chlorophyte peak. The abrupt transitions, within a relatively short time frame, may be an indication of increasing variability in algal communities relating to perturbation by eutrophication (Cottingham et al., 2000) (see also Carpenter and Brock (2006) and Scheffer et al. (2009) regarding the relationship between regime shifts and variability).

The mechanism behind the decrease in N_2 -fixers can only be surmised as the prevalence of N_2 -fixers in lakes, and their relationship to eutrophication, is somewhat ambivalent. Often, they are associated with situations where P is in relatively abundant supply, and N becomes limiting (e.g. Leavitt and Findlay, 1994; Vanni et al., 2011; McGowan et al., 2012). However, where N is limiting, N_2 -fixers can usually not compensate fully for this limitation (Lewis Jr. and Wurtsbaugh, 2008), and limiting concentrations as predicted by stoichiometry are not always associated with large proportions of N_2 -fixers (Reynolds, 1998; Paerl et al., 2011). Therefore, it cannot be concluded that N has become less limiting over time, especially as the modern limnological data set implies N limitation during summer. However, non- N_2 -fixers seem to become favoured with decreasing light penetration (Paerl et al., 2001; Havens et al., 2003), and the seasonally low secchi depths recorded in Hawes Water may lend support to light-related cyanobacterial community shifts. This could also involve a shift from benthic/epiphytic

to pelagic cyanobacteria, as for example, in the Arctic, echinenone and myxoxanthophyll have been found prevalent in benthic mats, and zeaxanthin primarily in the pelagic zone (Bonilla et al., 2005). Further, N₂-fixing cyanobacteria have been shown to be a major component of epiphytic communities (Cattaneo et al., 1998; Paerl et al., 2001), and epiphytic biomass to be higher on submerged than floating-leaved plants (Cattaneo et al., 1998). Increases in Nymphaeaceae indicate increases in floating-leaved biomass, and fossil *Simocephalus* (a filter feeder (Mangalo, 1987)) and *Pseudochydorus* (a scavenger, often feeding on other cladocerans (Fryer, 1968)), suggest increasing food abundance in the pelagic zone, cascading through the food web. Both patterns are in agreement with the idea of increasing pelagic productivity from the 1960s to the present day, combined with associated cyanobacterial shifts over the 1960s and -70s. Such a shift may also in part explain the decrease in mollusc abundance over the same time period, through a loss of both structurally complex habitat and epiphytes (food) (Cattaneo et al., 1998).

The abrupt microphyte shifts occur over a time frame encompassing decreases in macrophyte species richness as indicated by historical data. *P. crispus*, *P. friesii*, *P. obtusifolius*, recorded in 1915 and 1969 (Table 3.2, Figure 3.16) were not recorded in the early 1980s, when *E. canadensis* was first recorded, suggesting the Potamogetonaceae may have been outcompeted by *E. canadensis*. Regrettably, because *E. canadensis* does not produce remains that persist in the fossil record in the UK (Davidson et al., 2005), the timing of its invasion and increase in abundance can not be ascertained. From the 1980s to the 1990s, the colonisation depth of *C. rudis* reduced from 7 to 4 m, with declines also in the abundances of *Potamogeton lucens* and *Hippuris vulgaris*. This is in contrast to the increase in Nymphaeaceae as shown by macrofossil data, indicating significant changes in macrophyte community composition leading towards the end of the 20th century including a decrease in species richness.

Data from the end of the 20th century to 2009 give unequivocal evidence of shifts towards more pelagic productivity. Various limnological parameters were monitored as part of a late glacial/Holocene study between 1999 and 2000 (J. Marshall, unpubl.), providing an opportunity to compare data with this study and thus assess changes over the last decade. TP, SRP and NO₃-N data suggest in-lake concentrations were not very different from 2009/10 values, and stratification occurred at similar depths as currently. However, chl *a* data reveal more than a twofold increase in pelagic productivity over this period (maxima <10 µg L⁻¹ vs

25 $\mu\text{g L}^{-1}$). Chl *a* concentrations in 1999/2000 were low overall, and maxima were recorded close to the thermocline through the summer period in 1999 (Figure 3.18). These maxima were mirrored by the vertical concentration profile of oxygen, reflecting photosynthesis. Although the depth distribution of phytoplankton was not measured during 2009 and 2010, subsurface oxygen maxima were recorded, and it is therefore likely that subsurface maxima of phytoplankton also occur at present. Therefore, the more than twofold increase between 1999 and 2009/2010 may in fact be a very conservative estimate of the true increase in pelagic productivity.

Despite low overall phytoplankton abundance between 1999 and 2000, blooms in spring were dominated by the diatom *Stephanodiscus* sp. (J. Marshall, unpubl.). Further, in a core collected from Hawes Water in 2002, Bennion (2004) reported upcore increases in *Fragilaria crotonensis*, *Asterionella formosa* and *Stephanodiscus medius*. All the aforementioned species indicate nutrient enrichment (Reynolds et al., 2002). Therefore, it seems compositional changes in the plankton preceded the drastic increase in quantity. Importantly, the time frame encompassing the compositional and numerical change also encompasses the disappearance of *Chara rudis* from the site. In agreement with recent and relatively rapid ecological degradation, the uppermost levels of both HAWE3 and HAWE5 are the only records of *Daphnia* in Hawes Water, and the macrofossil data also indicate drastic increases in Nymphaeaceae, mirroring the current dominance of *N. lutea* in deeper water and a displacement of submerged macrophytes (Figure 3.16).

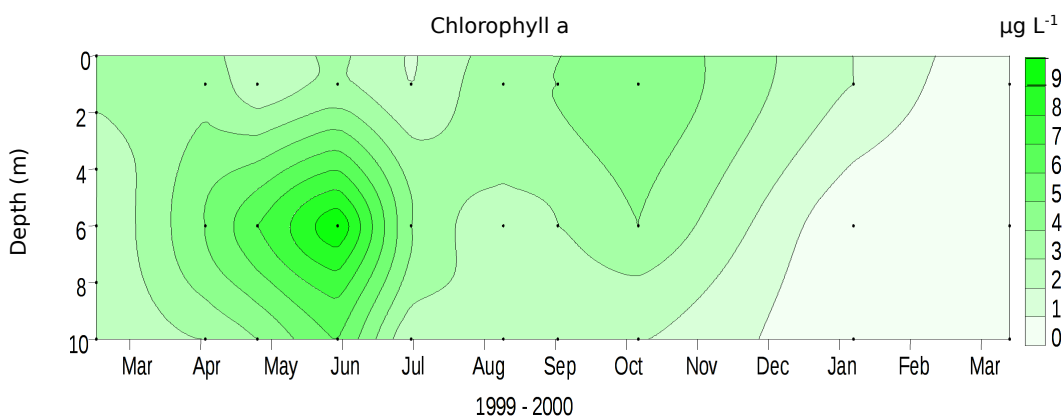


Figure 3.18: Contour map of the depth distribution of phytoplankton as chlorophyll *a*. Reproduction conditional on permission by J. Marshall.

The pigment data from the top section of the core do not indicate increased photoautotrophic

productivity (Figure 3.12). McGowan et al. (2005) explained similar patterns in two Danish lakes by shifts from benthic to pelagic pathways, and therefore qualitative rather than quantitative eutrophication responses. While such shifts certainly have taken place in Hawes Water, other factors could also influence sediment pigment concentrations. For example, pigment degradation during sinking through the water column can be very high (Sanger, 1988; Leavitt, 1993), and increases in overall productivity may therefore be masked by increased losses. Importantly, the large increase in water-column chl *a* concentrations between 1999 and 2009/2010 indicates that the increases in *Simocephalus*, *Daphnia* and *Pseudochydorus* in the core top section are unlikely to have suppressed the phytoplankton, thereby explaining decreases in core pigment concentrations. Rather, their abundance is a reflection of increased food availability. The fish communities in Hawes Water have not been monitored, preventing analysis of top predator responses to eutrophication.

Although the decline in species richness and diminished distribution of key species (*Chara*, *Potamogeton*) is a negative change, the large decline in the abundance of the invasive *E. canadensis* since the 1980s is favourable. This pattern cannot be causally linked to environmental conditions with certainty. Firstly, although the species is often associated with more eutrophic waters, it can also thrive in low-nutrient conditions (Søndergaard et al., 2010). Secondly, in Malham Tarn, a mesotrophic marl lake in Yorkshire, periodic inverse cyclicity in *Chara* and *Elodea canadensis* has been observed for several decades (Hinton, 2005), and relatively short-term changes in *E. canadensis* may not reflect a consistent trend. Finally, in the UK overall, invasions have often been followed first by a period of increase, then by decline and even extinction, including sudden population crashes (Department for Environment, Food and Rural Affairs, 2011), suggesting a degree of stochasticity and/or internal dynamics in abundance over time.

3.5.5 Light climate and changes in colonisation depth

In favourable conditions, charophytes are able to colonise great depths, and have been frequently observed growing below 10 m (e.g. Spence, 1982; Spence et al., 1984; Schwarz and Hawes, 1997; Pybus et al., 2003). In Loch Borrallie, a marl lake in Scotland, deep-growing charophytes include *C. contraria* (Spence et al., 1984), also recorded in Hawes Water and possibly forming part of the community on the coring site based on fossil oospore morphology

typing. Therefore, in pre-disturbance condition such as indicated by core data prior to the 1800s, Hawes Water could have supported macrophytes throughout the basin compared with only fringe colonisation at present (Figures 3.16, 3.19). As macrophyte growth is limited on steep slopes (Whiteside and Lindegaard, 1982; Duarte and Kalff, 1986), a reduction in colonisation depth to less than 8 m would have had an immense impact on the distribution (Figure 3.19) and composition of macrophytic communities (see e.g. Vestergaard and Sand-Jensen, 2000).

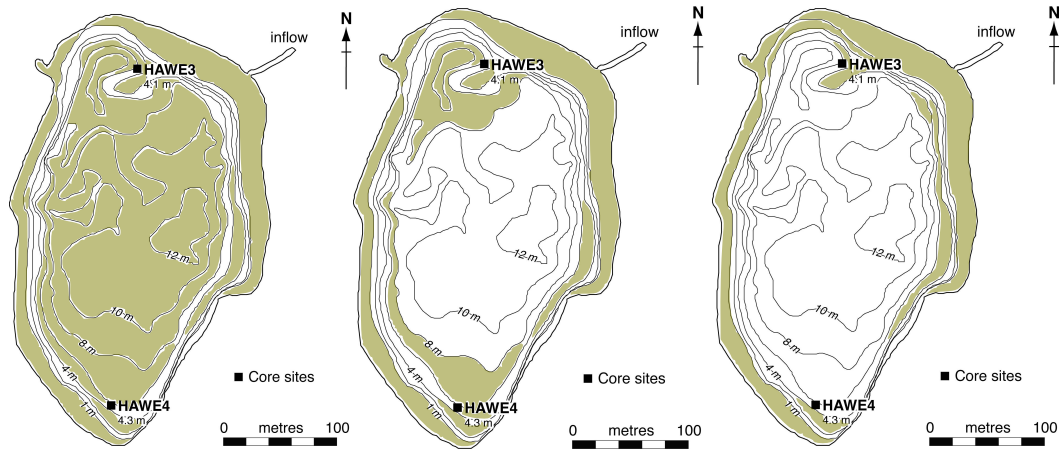


Figure 3.19: Map of hypothetical macrophyte cover, assuming avoidance of steep slopes. From left to right: Colonisation depth >12 m, as typical for charophytes in oligotrophic, clear lakes; Colonisation depth <8 m, up to early 1980s when *C. rudis* still grew to 7 m; Current colonisation depth, greatly restricting macrophyte distribution.

Schwarz and Hawes (1997) noted declines in charophyte biomass under average SI between 10 and 4.5, indicating that the boundary of 5 (Middelboe and Markager, 1997) used in this study for theoretical charophyte growth may be an overestimate, especially within depth limits of angiosperms that are strong competitors for light given sufficient nutrients. *Chara rudis* and *C. hispida* var. *major*, deep-growing charophytes still recorded in Hawes Water in 1999, have disappeared, while the deep-growing *C. contraria*, and *C. aspera*, commonly found between 2 and 4 m (Moore, 2005), are restricted to shallow open pockets among the reeds. Clearly, the distribution of charophytes in Hawes Water has become limited.

The decline also of the Potamogetonaceae is highly likely to have been brought on by decreasing water transparency, given the associated reduction in colonisable surface area (Vestergaard and Sand-Jensen, 2000) and the high potential depths of several *Potamogeton* species indicated

for marl lakes (Spence et al., 1984; Hilt et al., 2010). A diverse community of Potamogetonaceae and Characeae in the mid-1900s, when water transparency was high, is therefore very likely to have existed (Figure 3.16). Even further back in time in the early 1900s, as indicated by early macrophyte surveys, less dense reedswamp and negligible siltation would also have provided diverse habitat in the shallows.

3.5.6 Conclusion

Considering the relatively low surface water nutrient and chl *a* concentrations in Hawes Water, the ecological change that has taken place since at least the early 1800s is somewhat surprising and concerning. Reductions in species numbers and evenness, coupled with the extinction of the nationally near-threatened *Chara rudis* are relatively recent occurrences. As the sequence of eutrophication-related change shows signs of acceleration with time, it is highly likely that Hawes Water will undergo further ecological degradation in the near future. This also applies to the marginal charophyte populations, as they have declined most likely due to increasingly dense reed beds combined with tree encroachment (*Salix*, *Alnus*). Importantly, this study has shown that marl lakes are very sensitive to changes in nutrient loading, especially where water depth gradients give rise to large changes in macrophyte coverage with only small changes in light penetration. The apparent losses of propagule banks of perennial charophytes, dating as far back as two centuries ago, indicate the reproductive potential of charophytes is particularly sensitive to changes in light penetration. The low numbers of oospores in the recent sediment at the coring location will hinder re-establishment of charophytes even if limnetic conditions were favourable due to slow recovery of charophytes on sites with zero biomass (Schwarz et al., 1999) and observed dependence of recruitment success with propagule bank size (van den Berg et al., 2001). Further, while germination success may be independent of light conditions, this is not the case for germling survival (de Winton et al., 2004). Recolonisation after reductions in external nutrient loading may be a slow process in deeper marl lakes.

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4 Synchronous eutrophication-driven change in the biological structure of a small calcareous lake, Cunswick Tarn, UK

4.1 Abstract

Eutrophication has been identified as the single most important threat to highly calcareous (marl) lakes in the UK. Despite their unique chemical and biological characteristics and rarity as a lake type in the UK, comprehensive research relating to their ecological response to eutrophication is relatively scant. In order to comprehensively elucidate the timing, magnitude and characteristics of eutrophication-driven change in marl lakes, a multi-proxy palaeolimnological study, combined with a two-year limnological monitoring programme, was undertaken at Cunswick Tarn (UK), a small, eutrophic kettlehole marl lake. Palaeolimnological data reveal rapid and dramatic changes in the biological and chemical structure of the Tarn, with three major ecological phases. The pre-1900s were largely characterised by low overall production, especially in the pelagic zone, and a diverse macrophyte community. Early stages of eutrophication in the early 1900s involved sub-decadal changes including an increase in carbonate deposition, and the abundances of charophytes and plant-associated cladocerans. However, plankton were suppressed during this time, indicating high water transparency and food webs based predominantly on benthic productivity. Decreases in carbonate content upcore (post-1920s) are associated with an increase in floating-leaved macrophytes and pelagic cladocerans that continues through to the core top where cyanobacteria and planktonic diatoms become more prevalent. The timing of these changes suggest a relatively rapid transition from benthic to more pelagic productivity in synchrony with substantial changes in macrophyte and cladoceran species assemblages. The patterns displayed by macrophyte, microphytes and invertebrates show that marl lakes are sensitive to eutrophication, and that centennial-scale gradual change also includes more stepwise, punctuated phases.

4.2 Introduction

Marl lakes are highly calcareous lakes that lie in limestone, sandstone, chalk and machair catchments, where strata of impermeable rock trap water, or the water table is high (Pentecost, 2009). The dissolution of these rocks in the catchment results in high in-lake carbonate and calcium concentrations, resulting in high in-lake pH and alkalinity. Calcium carbonate (marl) is deposited onto the lake bed when the water column becomes supersaturated with

carbonate. This may occur as a result of photosynthesis driving increases in pH, high temperatures in summer lowering the carbonate saturation point, and/or carbon dioxide evasion where water supersaturated with carbon dioxide equilibrates with the atmosphere (see e.g. Pentecost, 1995). For a calcareous lake to be defined as a marl lake, an average surface-sediment calcium carbonate content exceeding 50% of the dry weight of the inorganic fraction has been suggested as a requirement (Pentecost, 2009), introducing a very specific link between lake typology and chemistry.

Importantly, the chemical characteristics of marl lakes structure their biological communities. Macrophytes and phytoplankton capable of using bicarbonate efficiently for photosynthesis are favoured (Lund, 1961; Spence, 1967; Allen and Spence, 1981; Reynolds, 1998), and high calcium concentrations permit high abundance of Mollusca (Dillon, 2000). In terms of their biology, marl lakes fall within category 3140 of the European Union Habitats scheme (EC-DG ENV 2007) as "hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp." A similar classification scheme has been developed specifically for British lake habitats, with marl lakes falling into category I, "base-rich lowland lakes, with *Chara* spp., *Myriophyllum spicatum* and a diversity of *Potamogeton* species" (Palmer et al., 1992; Duigan et al., 2007). Charophytes are macrophytic algae that typically form a large portion of the standing biomass in shallower marl lakes. Many marl lakes in the UK and Ireland have conservation designations. However, the ecological status of most of these lakes is either under threat principally from nutrient enrichment or poorly known, and a good understanding of their reference state as well as ecological eutrophication responses is desirable to inform management and restoration. This is of particular importance due to the decline of several charophyte species across Britain (Stewart, 2004).

It is thought that marl lakes may be naturally buffered against the adverse effects of eutrophication, since phosphorus can precipitate with calcite (Otsuki and Wetzel, 1972; House, 1990; Siong and Asaeda, 2006; Robertson et al., 2007). Charophyte stands in particular contribute to phosphorus immobilisation via several mechanisms. Firstly, calcite accumulates on thalli and oospores, and can persist throughout the winter months, trapping phosphorus in the long term. Secondly, charophytes often grow as dense stands, which stabilises the water column and reduces sediment (and nutrient) resuspension (Krolikowska, 1997; Hargeby et al., 1994; Blindow et al., 2002; van den Berg et al., 1998). Finally, by virtue of having the potential to

overwinter, and resisting decomposition, charophytes also limit the availability of nutrients beyond the main growing season, reducing the potential of phytoplankton growth (Kufel and Kufel, 2002; Krolowska, 1997; Siong and Asaeda, 2006). Some studies have found reduced nutrient concentrations and/or higher water clarity within charophyte stands compared to other lake areas (Krolowska, 1997; Meijer and Hosper, 1997; Steinman et al., 2002).

Various feedbacks may operate between nutrients, carbonate chemistry and charophytes, with interesting consequences for lake ecology. In theory, the charophyte-phosphorus limitation relationship is a positive feedback loop, as limiting nutrients available to phytoplankton furthers macrophyte persistence. However, marl precipitation is inhibited by various forms of organic matter as well as phosphorus itself (Reynolds, 1978; House, 1990; Grossl and Inskeep, 1992; Danen-Louwerse et al., 1995; Hoch et al., 2000). An abundance of either or both can potentially thwart coprecipitation and interfere with the negative feedback on eutrophication (Reynolds, 1978), leading to higher nutrient concentrations in the water column. Thresholds and rapid ecological responses may be expected in this scenario, as increases in phytoplankton trigger light limitation, causing charophytes to decline in favour of angiosperms (see for example Hargeby et al., 1994; Davidson et al., 2005; Ayres et al., 2008; Penning et al., 2008). A decline in charophytes reduces calcification and phosphorus trapping, leading to even higher potential abundances of phytoplankton. However, such a break point is naturally less likely in very shallow lakes where the effects of light limitation are less pronounced compared to deeper lakes, and charophytes may therefore persist at relatively high nutrient concentrations (Søndergaard et al., 2010).

Regardless of calcite precipitation, in the absence of other limiting factors, increased nutrient inputs inevitably lead to increased macrophyte as well as phytoplankton biomass and thus the accumulation of highly organic sediment. This can result in changes in redox conditions, increased anoxia and increased anaerobic respiration, leading to increased nutrient recycling (Jansson, 1987; Golterman, 2001). Such conditions may also be detrimental to macrophyte growth (Schutten et al., 2005), including charophytes (Smith, 2003; Sederias and Colman, 2009).

While there are studies on eutrophication and individual components relating to marl lake ecology, comprehensive long-term studies of marl lakes undergoing eutrophication are rare. This is particularly true of the typically decadal-centennial timescales encompassing change

driven by eutrophication. By combining multiple palaeolimnological methods and contemporary monitoring of a currently eutrophic marl lake (Cunswick Tarn, English Lake District), this study aimed to answer the following questions: 1) What biological communities characterised Cunswick Tarn before eutrophication? 2) What were, and are, the ecological responses of the site to eutrophication? 3) If major change has taken place, was it rapid or gradual?

Several hypotheses were developed. Given the steeply shelving depth profile of Cunswick Tarn, and the sensitivity of charophytes to light penetration, eutrophication effects were expected to be reflected by noticeable and relatively rapid changes in the charophyte community (mainly through numerical responses of fossil oospores mirroring the abundance of charophytes (Zhao et al., 2006). These changes would be associated with upcore increases in remains of tall-growing or floating-leaved angiosperms as well as diatoms, pigments and cladocerans reflecting increased pelagic productivity, and a clear transition from a predominantly benthic to an increasingly pelagic system (Vadeboncoeur et al., 2003). Also, if marl lakes are truly distinct from other lake types, calcium (carbonate) would be expected to be a key driver of biological community composition. If Cunswick Tarn has reacted to eutrophication in a similar fashion to other lake types, phosphorus would also be expected to contribute to such changes. Therefore, it was expected that both sedimentary phosphorus (P) and calcium (Ca) concentrations would exhibit strong relationships with the fossil data of biological communities.

4.3 Methods

4.3.1 Study site

Cunswick Tarn is a small lowland (0.8 ha, 138 m a.s.l.) kettlehole lake in Cumbria, UK (Figures 4.1 and 4.2). It has mean and maximum depths of 3.7 and 6.4 m, respectively. Large areas of shallow water are restricted to the northern and southern margins, as the eastern and western margins have steeper slopes. Most water is received from a stream at the southeast end, with minor contributions from small streams flowing off the wooded slope to the east (Figure 4.3). All streams are high in carbonates and precipitate marl, reflected by the high in-lake mean pH of 8.17. There is phosphorus- and organic-rich seepage from woodland and pastures to the north. A low-gradient, occasionally still outflow lies at the southwest end (Figure 4.3). The catchment is small and comprises mainly improved pasture, with woodland

to the north and east. The Tarn is sheltered and waves form only in strong winds. While the catchment has remained largely unchanged since at least 1850, the wooded slope has occasionally been deforested, and the northern bay has expanded over recent years due to erosion. Currently, the lake is used for duck shooting.

The lake is eutrophic, with current total phosphorus (TP) and chlorophyll *a* (chl *a*) means of



Figure 4.1: Cunswick Tarn from the north bank in August 2011.

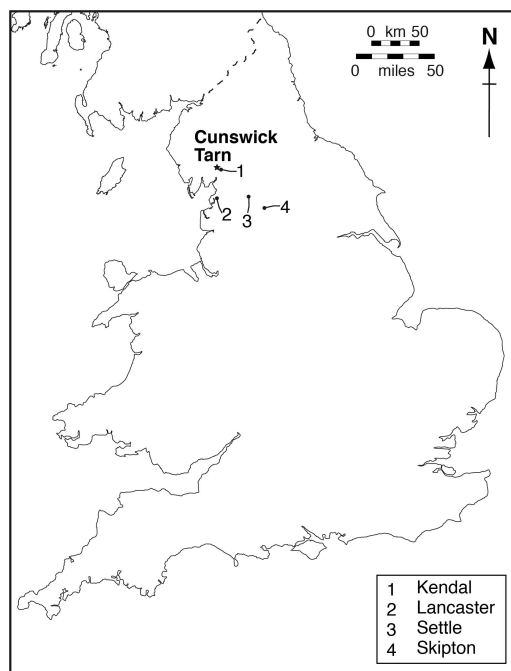


Figure 4.2: A map of the UK showing Cunswick Tarn.

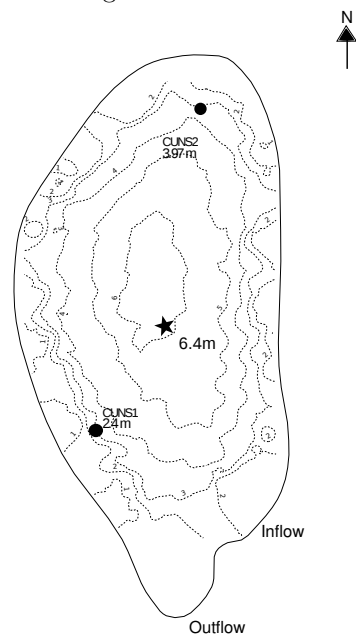


Figure 4.3: The bathymetry of Cunswick Tarn, showing the location and water depth of core recovery.

55.8 and 84 $\mu\text{g L}^{-1}$, respectively. A drain pipe emptying into the lake from the north pasture, grain deposited in the water, and the artificially large waterfowl population, all contribute to the high concentration of nutrients in the lake. The erosion at the northern bank results in locally highly turbid water and loose sediment. The accumulation of senescing biomass on the

lake bed and therefore decomposition-related respiration, combined with low wind stress and high epilimnetic productivity results in strong chemical stratification during summer reflected in abundant oxygen being limited to the uppermost 3-4 m (Figure 4.4).

The lake has low macrophyte diversity and is dominated by species typically associated

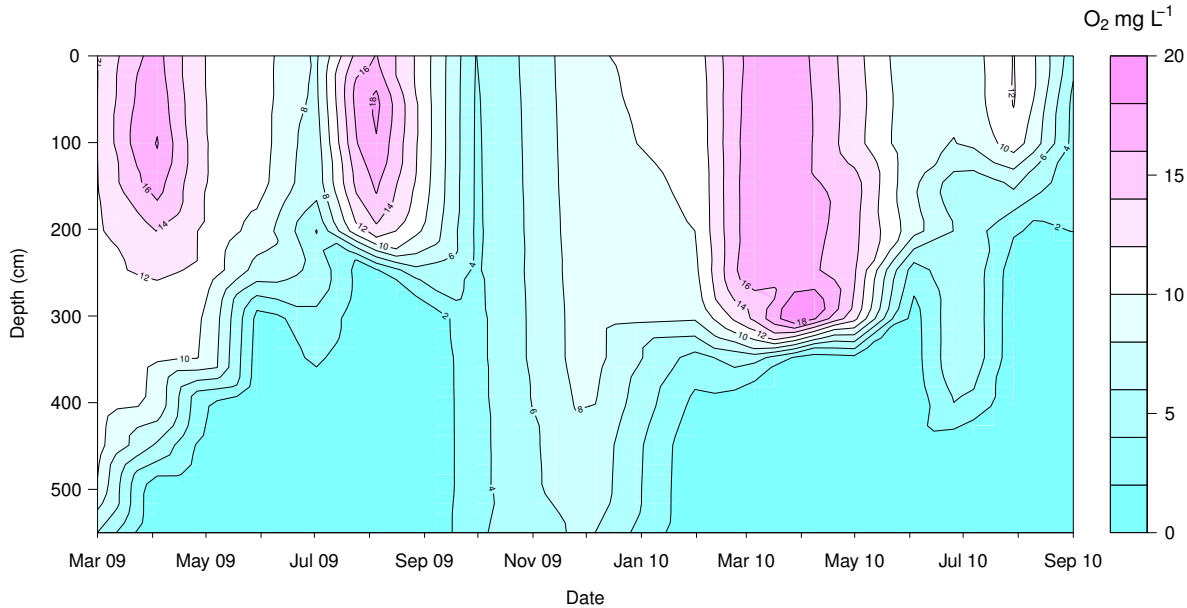


Figure 4.4: Depth profile of oxygen in Cunswick Tarn over 2009 - 2010.

with eutrophic conditions (*Nuphar lutea*, *Potamogeton pusillus/berchtoldii* and the invasive *Elodea canadensis*). In addition, dense floating mats of *Cladophora* sp. occur during summer. Charophytes are absent. With the exception of the margins by the eroding northern bank, macrophytes colonise all shallow areas and extend to depths of approximately 4-5 m. Phytoplankton are abundant during the spring and summer months, which is mirrored by pockets of high oxygen concentrations at the lake surface (Figure 4.4). Fish are not thought to be present in the lake as indicated by high abundances of large-bodied zooplankton and *Chaoborus flavicans* + *Chaoborus* cf. *obscuripes* in the plankton.

4.3.2 Core collection

Two littoral sediment cores (as defined by macrophyte colonisation depth) were collected from Cunswick Tarn (Figure 4.3). CUNS1 (74 cm) was taken in January 2008 at a depth of 2.4 m from the southwestern end of the lake using a “fat” Livingstone piston corer (internal diameter 71 mm). CUNS2 (94 cm) was taken in October 2009 from the northern margins at

a water depth of 4 m using a “Big Ben” piston corer (internal diameter 140 mm) (Patmore et al., *in prep*). Visible changes in sediment colour or structure in the cores were noted in the field. Both cores were extruded at 1 cm intervals, and subsamples for pigment analysis were immediately frozen.

4.3.3 Core chronology and lithostratigraphy

Core CUN51 was dated by spheroidal carbonaceous particle (SCP) analysis (Rose et al., 1995; Rose and Appleby, 2005). SCPs are artificially produced as the result of fossil fuel combustion at high temperatures, and therefore provide a signal of industrially derived atmospheric pollution.

Core CUN52 was dated radiometrically with ^{210}Pb , a naturally produced radionuclide derived from atmospheric fallout (termed unsupported ^{210}Pb) with a half-life of 22.3 years. Sediment ^{210}Pb concentration profiles can yield accurate stratigraphic dates for the past 150 years. ^{137}Cs (half-life 30 years) and ^{241}Am (half-life 432.2 years) are artificially produced radionuclides, deposited as atmospheric fallout from nuclear weapons testing and nuclear reactor accidents, and were used as markers to secure the chronology indicated by ^{210}Pb data.

Freeze-dried sediment samples were analysed for ^{210}Pb , ^{226}Ra , ^{137}Cs and ^{241}Am by direct gamma assay in the Bloomsbury Environmental Isotope Facility (BEIF) at University College London, using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra using the 295 keV and 352 keV gamma rays emitted by its daughter isotope ^{214}Pb following three weeks of storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 keV and 59.5 keV (Appleby et al., 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby et al., 1992).

Carbonate and organic matter content were quantified using the loss on ignition (LOI) procedure following Dean (1974). Weights after combustion were recorded as percentages of dry weight. For percentage carbonate, the weight lost was first multiplied by 1.36 to account for the difference in molecular weight of CO_2 and CO_3^{2-} .

Core CUN2 elemental composition was analysed by X-ray fluorescence (XRF) (Boyle, 2002). Every cm for the uppermost 10 cm, and every two cm thereafter, were analysed. Samples of approximately 1 (+/- .009) g of sediment were weighed out, freeze-dried and ground to homogeneity (Jansen et al., 1998), thereafter run on a Spectro X-Lab 2000 in random order with two standards.

4.3.4 Pigments

Pigments were analysed for all CUN2 sediment intervals at the University of Nottingham laboratories following McGowan et al. (2012). All samples were wrapped in foil to prevent photodegradation, and dried with an Edwards Modulyo 4k freeze-drier prior to analysis, and kept frozen in the dark thereafter. Samples were extracted overnight at 4°C in a mixture of acetone, methanol and water (80 : 15 : 5). Extracts were filtered with a 0.22-µm PTFE filter, dried under N₂ gas and redissolved in a 70 : 25 : 5 mixture of acetone, ion pairing reagent (IPR 0.75 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 mL water) and methanol. Thereafter, analysis was undertaken using an Agilent Technologies 1200 Series high-performance liquid chromatographer (HPLC) fitted with a Thermo Scientific ODS Hypersil reverse phase column (205 x 4.6 mm, 5 µm particle size). An injection volume of 100 µL was used. Analysis followed the method outlined in Chen et al. (2001), with slight modification (Table 4.1). Between 0.1 and 0.4 g of freeze-dried sediment was required to attain sufficient colour for analysis. All samples were analysed in random order and kept in low light conditions at all times. HPLC solvents were degassed before use (Decon[®] FS200b sonicator). All runs included a green standard derived from grass, serving the purpose of showing the retention times of key pigments, and were calibrated against commercial standards to convert peak areas to concentrations.

In August 2011, two contemporary water samples were collected from depths of 4.5 and 5.5 m using a van Dorn sampler in order to establish presence of photosynthesising bacteria in the meta/hypolimnion. The water was filtered through 1.2 µm pore size Whatman[™] GF/C filters, and filters were subsequently wrapped in foil to exclude light. Filters were frozen after collection and kept in the dark, and were delivered in person to the University of Nottingham for analysis following the procedure outlined above.

Pigments were identified by comparison with the absorption characteristics and elution times

Table 4.1: Modifications of the HPLC solvents used by Chen et al. (2001). A: 80% methanol 20% 0.5 mol ammonium acetate, B: 90% acetonitrile 10% deionised water, C: HPLC-grade ethyl acetate

Time (min)	A	B	C	Flow mL min ⁻¹
0	100	0	0	1
4	0	100	0	1
38	0	25	75	1
39	0	25	75	1
43	100	0	0	1
52	100	0	0	1

of standards. Echinenone coeluted with chlorophyll *a* and therefore all chlorophyll *a* concentrations are partly biased. However, the contamination should not be significant as assessed by looking at individual spectra. In addition to frequently encountered pigments, there were two unidentified carotenoid pigments, one of unknown photoautotrophic bacterial origin (referred to as “bac”), and one likely to be from purple sulfur bacteria (referred to as “cf. okenone”). The identified pigments were divided into groups such that total primary production is indicated by the generic pigments chlorophyll *a* and its degradation products as well as β -carotene; cryptophytes are indicated by alloxanthin; cyanobacteria by canthaxanthin and zeaxanthin, and chlorophytes by lutein, chlorophyll *b* and pheophytin *b* (McGowan et al., 2005). The pigment chlorophyll *a*’ refers to the oxidative degradation product divinyl chlorophyll *a*..

Pigment preservation was regarded satisfactory due to presence of the degradation-prone fucoxanthin throughout the core. However, levels from the uppermost 4 cm were removed from analysis and diagrams due to rapid short-term post-depositional degradation as indicated by particularly large ratios of chlorophylls *a* and *b* to their degradation products. Changes in pigment concentrations further downcore were interpreted as independent of preservation effects where these ratios were relatively unchanging.

4.3.5 Diatoms

Diatom analysis on CUNS2 was undertaken using standard methods as outlined in Battarbee et al. (2001) (Stone, 2012). Diatom slides were analysed at two cm intervals, but the uppermost two samples were both analysed. Due to poor preservation, samples below a core depth of 45 cm were not counted. However, above this level preservation was satisfactory and no differential dissolution was observed with the exception of two samples, 35.5 and 19.5 cm (Stone, 2012). Abundances of epiphytic, benthic and pelagic diatoms were calculated such

that for species found in multiple habitats, abundance was accordingly divided.

4.3.6 Subfossil cladocerans and Chaoboridae

Preparation of subfossil cladoceran samples followed the principles outlined in Korhola and Rautio (2000). Approximately 1 g of sediment was analysed at 3 cm intervals from CUN2. Due to high concentrations of carbonate in the sediment, samples were treated with 10% HCl prior to 10% KOH and deionised water. All samples were sieved to avoid damage that can be caused by centrifugation. Each sample was divided into size fractions retained by meshes of 150 and 45 μm (Davidson et al., 2007). Counting was performed using both a Lund cell (smaller remains) and a Sedgwick rafter cell (larger remains) up to a minimum count of 300 individuals as given by the most abundant remain for each species. Where this number was not attained owing to scarcity of remains, counting was stopped at the total number of individuals in an entire sample (large fraction), or upon having counted more than 50% of a sample (small fraction). The proportion of sample analysed was estimated based on the difference in original sample weight before and after analysis. Data are expressed as the number of individuals per g of wet sediment, and as relative abundance to display key changes in community composition, in which case Chaoboridae are omitted to retain taxon specificity.

Not all remains could be assigned to a species. Post-abdominal claws may represent several species including *Ceriodaphnia* and *Daphnia* spp., both of which were present in the core. *Daphnia pulex*, on the other hand, due to additional smaller spines at the base of the claws, were identifiable. *Chydorus* remains were variable in size, which can be attributed to morphological plasticity within the species. All these remains were identified as *Chydorus sphaericus sensu lato*. Small *Bosmina* at the bottom of the core were in fragments, and mainly represented by antennules. While it is certain that *Bosmina longirostris* was present, absence of other species cannot be assumed and therefore these remains are referred to as *Bosmina* sp(p).

Identification was performed using Frey (1965), Flößner (2000) and Szeroczyńska and Sarmaja-Korjonen (2007). Larval *Chaoborus* spp. mandibles were identified with the aid of Aitken (1954); Uutala (1990); Živić and Marković (2006) and Palm et al. (2011); *Chaoborus obscuripes* and *Chaoborus crystallinus* were not separated and are referred to as *C. obs/crys*. No descriptions of the larval mandibles of *Chaoborus pallidus* were found, however, the species is assumed to be absent from the Cunsick Tarn record as the species is recorded only in

southern England (UK National Biodiversity Network - various contributors).

4.3.7 Macrofossils

Macrofossils of macrophytes, Cladocera, Trichoptera, algae, Bryozoa and Mollusca were enumerated at 6 cm intervals for core CUN2. CUN1 had been analysed for macrofossils previously (Bennion et al., 2009), and initially levels were chosen to complement these data to yield 21 samples at evenly spaced intervals. However, inconsistencies were found regarding the counts of certain smaller fossils, leading to the omission of the pre-existing data from diagrams. Analysed levels are therefore more unevenly distributed and total 16 in number.

Approximately 30 cm³ of wet sediment was used for full analysis for each sample of both cores. Additionally, in order to capture more information of species generally underrepresented by fossil remains (Zhao et al., 2006; Madgwick et al., 2011; Patmore et al., *in prep*), an additional 70 cm³ of sediment was analysed for rarer remains in core CUN2, yielding a total volume of 100 cm³. Sample volumes were determined by water displacement. Samples were soaked in 5% KOH overnight in order to break down sediment flocs, followed by sieving through meshes of 125 µm and 355 µm. The smaller mesh was not used for sieving the 70 cm³ sample of CUN2 sediment, as the rarer remains sought for were large enough to be retained on the coarser mesh. Counting was undertaken under a Wild M3Z dissecting microscope. All material of the larger size fraction, and a homogenous subsample of approximately 15% of the smaller size fraction, was analysed. All macrofossil counts are expressed as numbers per 100 cm³ wet sediment. Macrofossils counted as halves (for example ephippia, statoblasts) are expressed as the minimum number of individuals, and for practical purposes, CUN2 ephippia data are shown with CUN2 subfossil cladoceran data.

Macrofossils were identified using a dedicated reference collection of plant and animal parts and relevant publications held at UCL Geography. Where species-level detail was not attainable, remains represent species aggregates. These include *Daphnia hyalina* agg. ephippia and *Potamogeton pusillus/berchtoldii* leaf tips. Trichosclereids (leaf cells) of *Nymphaea alba* and *Nuphar lutea* were grouped as Nymphaeaceae. Not all molluscan fossils were identifiable due to breakage. Therefore counts of species generally reflect extrapolation from the ratio of entire identified individuals. For CUN2, molluscs were mostly identified to family or genus level only. Charophyte oospores are very difficult to identify to species level due to large mor-

phological variability and they were therefore aggregated. However, the relative abundance of different oospore morphotypes was noted and rough identification of selected specimens was performed using the UCL reference collection and an identification key (Haas, 1994).

4.3.8 Historical macrophyte records and community composition reconstructions

Species data on past macrophyte presence and distribution (early 1900s to present) were collated from records held by UCL and Natural England, and also by personal communication and journal/image searches on the web. These data, combined with the contemporary and palaeolimnological surveys conducted as part of this study and knowledge of community composition and zonation in comparable marl lakes (Chapter 5, Spence et al. 1984; Pentecost 2009; Hilt et al. 2010) or lakes with similar species (Spence, 1967, 1982), and books of charophytes (Moore, 2005) and Potamogetonaceae (Preston, 1995) were used to produce diagrammatic reconstructions of the macrophyte communities and colonisation depths in Cunswick Tarn for the present, the early 1900s, and the pre-1800s. Benthic/macrophytic and pelagic zooplankton were also portrayed on the basis of data from subfossil cladoceran and macrofossil analysis.

4.3.9 Data analysis

All data analysis was performed with R version 2.12 (R Development Core Team, 2010) using packages analogue (Simpson, 2007; Simpson and Oksanen, 2011), vegan (Oksanen et al., 2011), pcurve (Hastie et al., 2011), ade4 (Dufour and Dray, 2007) and lattice (Sarkar, 2008). Because the macrofossil analysis of CUNS1 was undertaken at relatively low resolution, and at intervals not matching the other proxies, these data are treated qualitatively only and the following section refers exclusively to core CUNS2 data.

To stratigraphically match LOI and XRF data with data derived for various biological proxies, missing data points of the former were approximated by averaging over consecutive data points. Due to the large fluctuation in core carbonate content and thus dilution by Ca of other sediment components in the XRF data, all elements other than Ca were divided by organic matter content. Although pigment data analyses were performed on both the complete data set (every cm) and that on the subset matching the frequency of subfossil cladoceran analysis

Table 4.2: Multivariate methods and transformations applied to selected biological proxies. “NA” indicates method was not performed for given proxy. All linear methods require Euclidean distances, but where such restrictions do not apply, the applied distance measure is given such that “Eu” = Euclidean and “BC” = Bray-Curtis. See text for full explanation.

Method	Transformation; Distance			
	Diatoms	Pigments	Cladocerans	Macrofossils
PCA	NA	$\ln(x + 1)$	Hellinger	$\ln(x + 1)$
RDA	NA	NA	Hellinger	NA
NMDS	NA	$\ln(x + 1)$; BC	Wisconsin($\sqrt{x/Y_{i+}}$); BC	$\ln(x + 1)$; BC
PCuA	Wisconsin($\sqrt{x/Y_{i+}}$); BC	$\ln(x + 1)$; BC	Wisconsin($\sqrt{x/Y_{i+}}$); BC	$\ln(x + 1)$; BC
Ward	NA	$\ln(x + 1)$; BC	$\sqrt{x/Y_{i+}}$; Eu	$\ln(x + 1)$; BC

(every 3 cm), only results of the latter are shown in ordination plots for simplicity, as this had no effect on the plot patterns. Data of ehippia were removed from the macrofossil data set in order not to duplicate community change shown by the cladoceran data set. *Daphnia* spines were removed from the subfossil cladoceran data set in order to avoid duplicate representation of the genus (post-abdominal claws were retained), as were the *Chaoborus* data in order to maintain taxon specificity.

4.3.10 Cluster analysis

Cluster analysis was performed to compare *a priori* carbonate zone boundaries with those statistically derived for community assemblages and to compare the divisions of clusters across the biological proxies. Diatom data were not included due to the brevity of the data set, and pigment data were subsetting (every three cm) to match core levels analysed for cladocerans for simplicity. Ward linkage was chosen for all proxies under transformations given in Table 4.2. To comply with the requirements of Ward linkage, the Bray-Curtis dissimilarity matrices of pigment and macrofossil data (plant and animal excluding cladocerans) were transformed into Euclidean matrices (Dufour and Dray, 2007). Further, both McQuitty and Ward linkage on the Bray-Curtis dissimilarity matrix of macrofossil data were performed for comparison, and both methods produced the same result, implying that for these data Ward linkage is a robust method despite fundamentally non-Euclidean distance matrices.

4.3.11 Unconstrained ordination and principal curves

Palaeoecological data are often coenocline, and species responses along ecological gradients are often variable, and therefore, requirements for linear methods (e.g. multivariate normality)

are often not met in addition to problems arising with Euclidean distances on successional data (Legendre and Gallagher, 2001). This was true for CUN2 data. To enable more ecologically meaningful ordinations, Hellinger distances (Equation 6), acquired by Hellinger transformation (Equation 7) followed by calculation of Euclidean distances, was applied to the cladoceran data set before applying linear methods (Legendre and Gallagher, 2001). This transformation is not applicable to data where columns represent different units or have uncomparable abundances resulting in misleading proportional data. Consequently, pigment and macrofossil data were log-transformed (Table 4.2) and non-linear ordinations using Bray-Curtis dissimilarities were preferred. PCAs were performed in order to project species data in ordination space.

$$D_{\text{Hellinger}}(x_1, x_2) = \sqrt{\sum_{j=1}^p \left(\frac{y_{1j}}{y_{1+}} - \frac{y_{2j}}{y_{2+}} \right)^2} \quad (6)$$

$$y'_{i,j} = \sqrt{\frac{y_{ij}}{y_{i+}}} \quad (7)$$

Where non-metric methods were used on relative data sets, Wisconsin transformation was also applied (Table 4.2) (Oksanen et al., 2011). This involves dividing species by their maxima, and sites by site totals, serving to reduce the range of values in the data set (though for relative abundance data only the species transformation will have an effect). Wisconsin transformation was prevented for the log-transformed data sets as in this case standardisation by rows was specifically avoided. For all NMDS analyses, Bray-Curtis dissimilarities were used.

Principal curves analysis (PCuA) is an alternative to linear and non-linear ordinations and particularly suitable for data sets with one long dominant gradient and variable and/or non-linear species responses (Hastie and Stuetzle, 1989; De'ath, 1999). PCuA uses the axis scores of other ordinations (commonly NMDS or PCA axis 1 scores) as a starting point and fits a curve through the points such that distances of the sums of squares are minimised. Species responses are estimated by smoothers (lowess, binomial general additive model (GAM), poisson GAM, spline) in the process, and degrees of freedom can be specified to control potential over- or underfitting (i.e. smoothing to noise rather than pattern, or conversely, smoothing to uninformative pattern). The adequacy of these parameters can be assessed by plotting the species data and the response curves fitted by the model.

NMDS axis 1 scores attained by using Bray-Curtis dissimilarities were used for all data sets as a starting point for principal curves (PCs), and smoothing splines were used to model species responses. Prior data transformations are shown in Table 4.2. Due to the large heterogeneity in the diatom data set, species response curves were allowed varied smoothers. Degrees of freedom were internally calculated for the pigment, diatom and cladoceran data as part of the function call. However, this did not perform adequately for the macrofossil data and the degrees of freedom were increased to 7 in order to capture more of the variation in the data (De'ath, 1999).

4.3.12 Constrained ordination

In order to test the preliminary hypotheses of the study relating to the relationship between both eutrophication (P) and marl precipitation (Ca), and biological communities, constrained ordination on CUN2 cladoceran data was performed using XRF Ca and P data. Fe was also included as a potential indicator of sedimentary redox changes. The performance of linear and non-linear methods was compared by passing cladoceran data to RDA and NMDS using Euclidean dissimilarities (Table 4.2). To further explore the relationship between the constraining variables and the data, CCA using both weighted average (WA/site) and linear constraint (LC) scores was performed. Both results were remarkably similar and therefore Fe, Ca and P were deemed suitable to describe variability in the cladoceran communities. The significance of each variable in capturing patterns in the cladoceran data was tested by random permutations of the vector fitting results on the NMDS ordination. The linearity of the gradients in ordination space was explored by plotting concentration gradients of the elements as contours (Oksanen et al., 2011).

4.4 Results

4.4.1 Lithostratigraphy and core chronologies

Similar changes in sediment colour and composition were evident for cores CUN1 and CUN2. In CUN1, from the core base to 55 cm, the sediment was dark in colour with scattered mollusc and *Chara* spp. stem remains. From 55 to 44 cm, the colour was lighter brown and higher in carbonates, with a slightly higher abundance of stem remains and molluscs. A light grey band interrupted the sequence between 44 and 40 cm, followed again by light brown sediment to 32 cm. Mollusc and stem remains were abundant throughout these

transitions. Above 32 cm, no stem encrustations were recorded, and sediment colour became darker. Mollusc remains were recorded to a depth of 17 cm upcore of which no remains were visible. CUNS2 sediment was distinctly clay-like between the core base and 83 cm. Upcore, to 42 cm, sediment was more coarse in texture and brown in colour. A sharp transition to more mollusc-rich material and lighter sediment colour occurred at 42 cm, marking a band of undefined dimensions due to smearing. Similar sediment characterised the core to 13 cm where sediment colour became markedly dark brown following through to the core top.

Colour changes in the two cores broadly correspond to changes in carbonate content (Figure 4.5), which exhibit three relatively distinct lithostratigraphic zones at similar depths. In CUNS1, carbonate concentrations between the core base and 42.5 cm are relatively high, fluctuating around 20% with the exception of a single peak exceeding 30%. In the corresponding section of CUNS2, carbonate content is much lower ($< 10\%$) and stable. In both CUNS1 and CUNS2, carbonate concentrations increase dramatically above 42.5 cm. Between 42.5 and 26.5 cm in CUNS1, and 42.5 and 22.5 cm in CUNS2, carbonate concentrations are similarly high. There are two separate concentration maxima, at 34.5 (43.8%) and 38.5 cm (43.7%) in CUNS1, and 26.5 (40.2%) and 36.5 cm (35.5%) in CUNS2. Upcore of 34.5 (CUNS1) and 26.5 cm (CUNS2), carbonate concentrations decline rapidly and fluctuate around 10 and 15% in CUNS1, and 15 and 25 % in CUNS2. Therefore, while CUNS1 sediment contains more carbonate below and within the core sections of abundant carbonate, the relationship is reversed towards the core top.

Core chronologies of CUNS1 (not shown) and -2 are similar within the uppermost 20 cm. Below this, in the region of fluctuating carbonate content, CUNS1 core levels are consistently assigned older dates than matching levels of CUNS2, with differences increasing to the extent of exceeding the limits of age model errors. Changes in core carbonate concentration add uncertainty to sediment dating because increases result in the dilution of all other sediment components. If this is not accounted for, sediment levels are assigned erroneous dates. This may be the case with CUNS1 as no adjustment to core carbonate change was made (N. Rose, pers. comm.). Further, the carbonate profiles of both cores show distinct and similar patterns at closely matched sediment depths. Given this match, the dating results of CUNS1 are disregarded and the CUNS2 dating profile is applied to both cores based on the carbonate profile (Handong Yang, pers. comm.).

Owing to non-monotonic variation in unsupported ^{210}Pb activity, the dating of CUNS2 followed the CRS model (Appleby, 2001). The depth at which the ^{137}Cs profile places the 1963 nuclear weapons testing peak is in slight disagreement with that of the CRS model. Because the ^{137}Cs peak is well-resolved, the chronology was adjusted accordingly. The oldest date assignable within reasonable error limits is 1867 (± 17 yrs), placed at a depth of 46.5 cm. There is an area between approximately 23.5 and 42.5 cm, coincident with changes in % carbonate, where estimated sedimentation rates increase dramatically and are accompanied by high error margins. This encompasses the end of the 19th and beginning of the 20th centuries. At approximately 22.5 cm, sedimentation rates decrease, stabilising at approximately $0.04 \text{ g cm}^{-2} \text{ yr}^{-1}$ with a slight increase in the uppermost 10 cm. These changes correspond to the early 1920s and 1980s respectively. Due to high error margins, no data have been expressed in terms of accumulation rate.

Sedimentation rates below the 1867 core level are not stable. Dates for key horizons below this level, determined by extrapolation, are therefore considered under extreme caution. A constant average sedimentation rate of 0.05 cm yr^{-1} , slightly higher than the minimum estimate, would date the sediment around 75 cm between the 11th and 15th centuries, and the core base between the 6th and the 11th century.

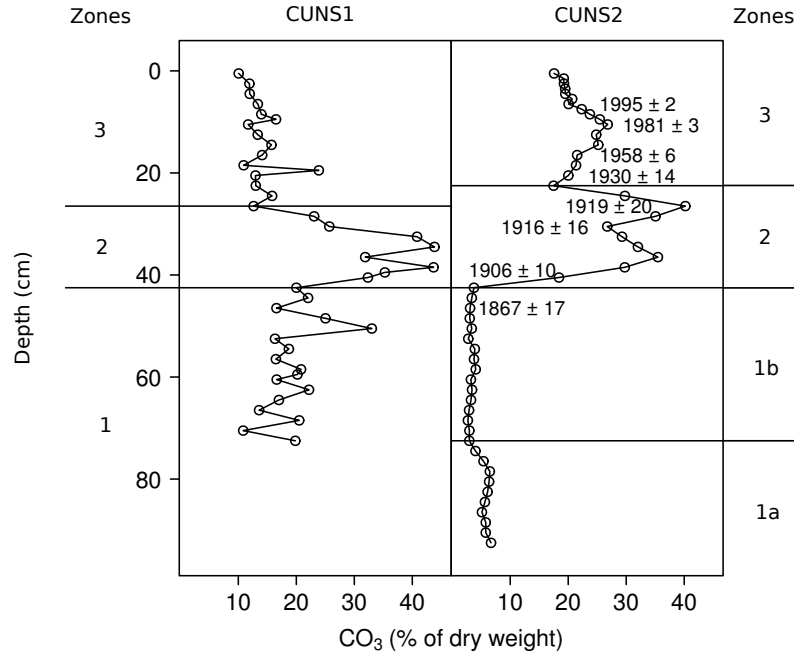


Figure 4.5: Carbonate concentrations in cores CUNS1 and CUNS2, shown with calendar years obtained by ^{210}Pb -dating of CUNS2 with errors in parentheses. (Radiometric analyses by Handong Yang)

The metal elements in CUNS2 sediment can generally be divided into those that increase upcore of 42.5 cm, coincident with the increase in carbonate concentration, and those that do not (Figure 4.6). Metals such as Sn, Ca, Pb, Cd, S, Zn, Cu and Na and importantly P belong to the former, P being the only element showing a consistent increase towards the core top. Certain metals show a distinct decline within the uppermost 10 cm, including Fe, Mg, Al, Ni, Ti and Si (Figure 4.6).

CUNS2 P concentrations are not positively correlated to Mn concentrations, and the highest P concentrations plot at intermediate Fe:Mn - low [Fe] regions (Figure 4.7), patterns inconsistent with redox-mediated P release (Mackereth, 1966; Hobbs et al., 2005).

4.4.2 Core zonation

While statistically independent assignment of zones in palaeoecological studies may mirror the “reinforcement syndrome” (Birks and Birks, 2006), zonation of multi-proxy data renders interpretation and discussion more user-friendly. As cores CUNS1 and CUNS2 display comparable changes in carbonate concentration, suggesting these changes were lakewide, and multiple palaeoecological data sets are used, this approach is appropriate and core data are discussed in terms of the three identified zones in the carbonate profiles. Instead of assignment based on absolute content, the zones were chosen to divide the cores into areas of stability and change, with emphasis on CUNS2 for which more analyses were performed. Consequently, zone 1 is characterised by relatively low carbonate content and minor fluctuations; zone 2 is characterised by dramatic increase, fluctuation and decrease, and zone 3 is characterised by lesser fluctuation and lower carbonate content. These patterns are broadly speaking reflected in all biological proxies (Section 4.4.4), which makes this approach particularly useful for this data set. The zone limits for CUNS1 are core base to 42.5 cm, 42.5 to 26.5 cm, and 26.5 cm to top, and for CUNS2 core base to 42.5 cm, 42.5 to 22.5 cm, and 22.5 cm to top. A subdivision of zone 1 (a and b) was deemed appropriate for CUNS2 based on clear changes in biological communities (Section 4.4.4) and slightly elevated carbonate below 72.5 cm .

4.4.3 Changes in biological communities

Zone 1 (c. 11th - 19th centuries); Core bases to 42.5 cm *Zone 1a* - Core CUNS2

Zone 1a is generally characterised by relatively low and invariable pigment concentrations

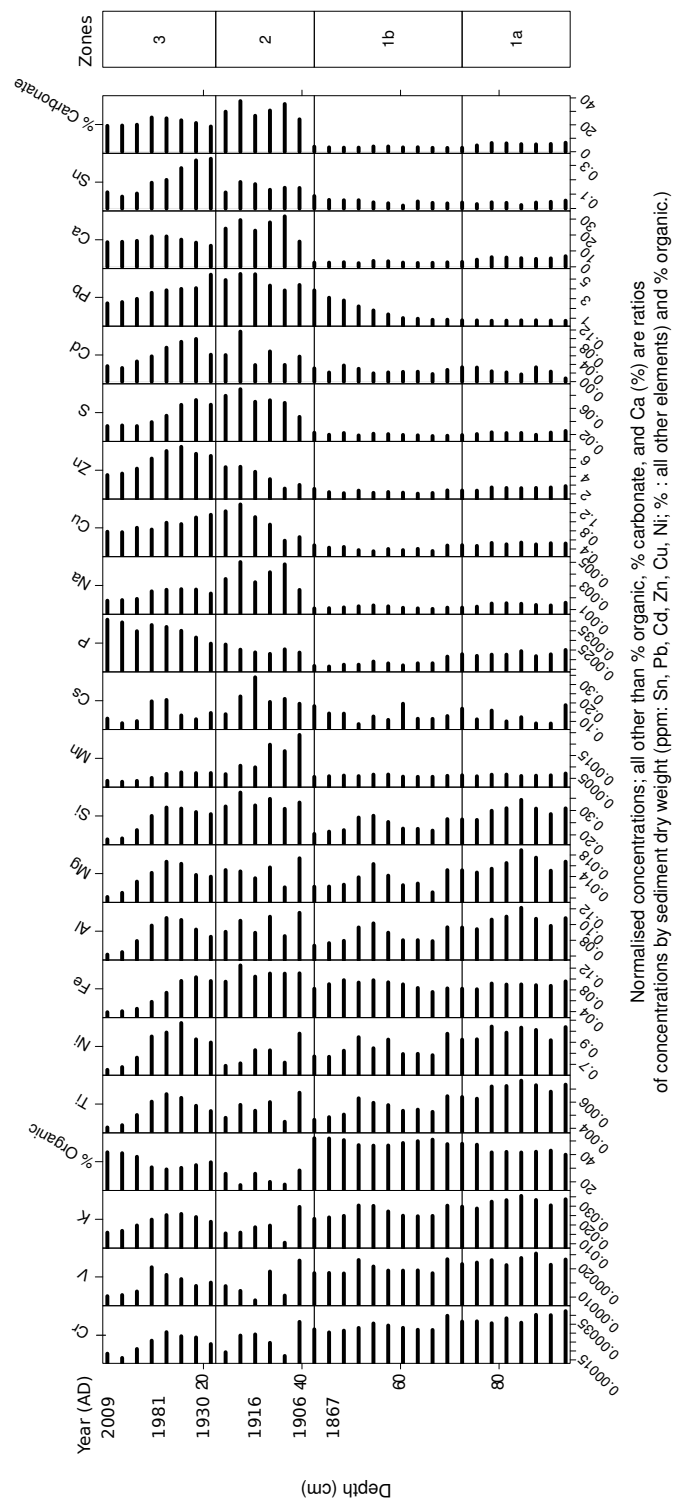


Figure 4.6: XRF data of CUN2 with all data other than Ca, % organic and % carbonate divided by % organic

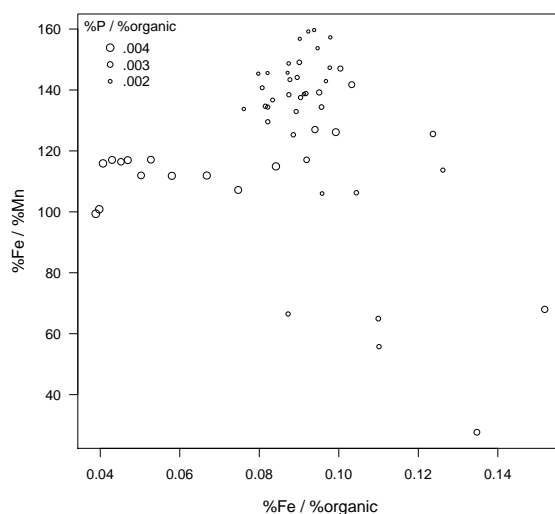


Figure 4.7: A biplot of core CUN2 Fe:Mn against Fe/%organic, with the size of the circles reflecting P concentrations, also normalised to organic content. The original percentages of elemental concentrations are expressed against dry weight of bulk sediment.

(Figure 4.9). However, relatively dramatic change occurs in cf. okenone concentrations, initially high between the core base and 80.5 cm, then declining rapidly upcore and disappearing at 72.5 cm. Pigments indicative of chlorophytes (lutein, chlorophyll *b* and pheophytin *b*) show small peaks from 85.5 - 80.5 cm, mirrored by the pattern of generic pigments (chlorophyll *a* and degradation products, β -carotene), suggesting overall abundance is driven by chlorophytes. Zeaxanthin and alloxanthin are most abundant from the core base to 80.5 cm, decreasing slightly towards the top of the zone, while canthaxanthin shows no clear trend. Canthaxanthin and zeaxanthin probably reflect different sources, and therefore variation in cyanobacterial community composition. Fucoxanthin is present at very low concentrations at the core base and increases towards the end of the zone, whereas diatoxanthin remains largely unchanged throughout the zone. Again, this probably reflects variable community composition of siliceous algae, with decreasing proportions of diatoms towards the end of the zone. Unfortunately, diatom analysis was not performed for core levels within this section (Figure 4.10). The unidentified bacterial pigment is most abundant at the base, but shows no overarching pattern.

Remains of pelagic cladocerans are relatively abundant in zone 1a, with maximal within-zone abundance at the core base (Figures 4.11, 4.12). This pattern is driven by the abundance of small *Bosmina longirostris*/spp., absent in other zones with the exception of the core top,

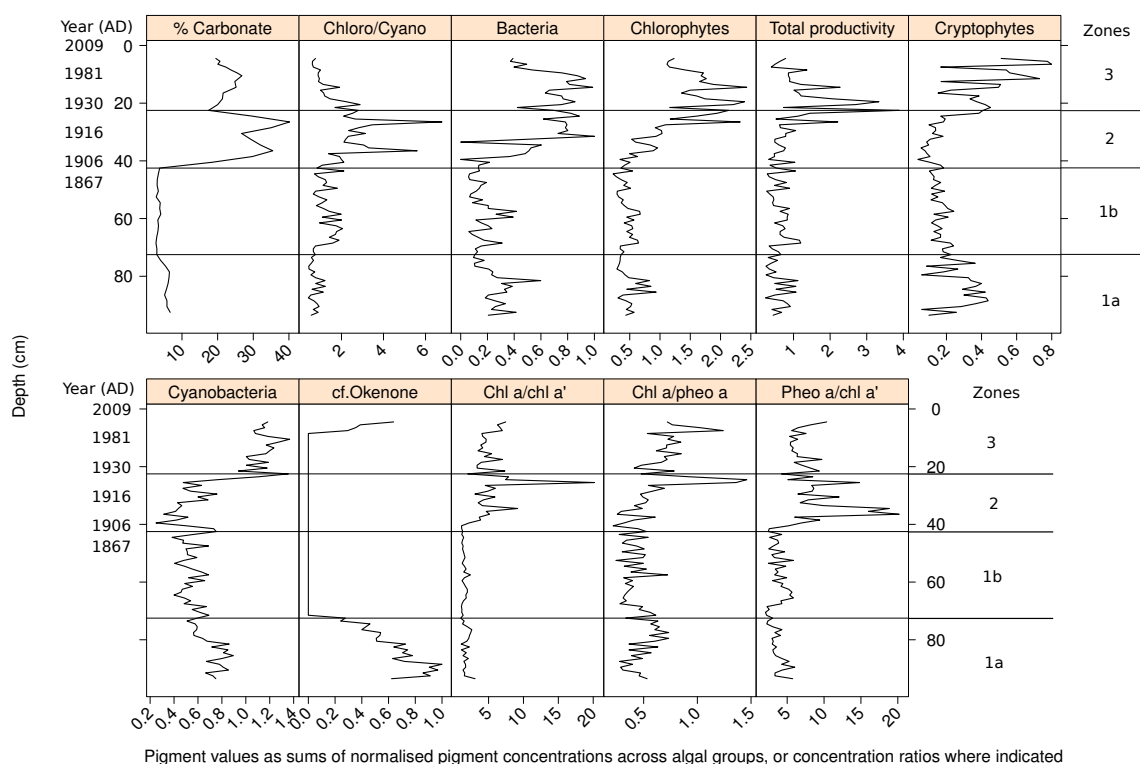


Figure 4.8: CUNS2 pigment data as normalised total abundance by taxonomic group. The peaks in the ratio between chlorophytes and cyanobacteria correspond to the peaks in core carbonate concentration. The outlying zero value of the bacterial pigment “bac” in zone 2 has no explanation.

where two individuals were found. Ehippia of *Daphnia* and *Simocephalus* spp. are also relatively abundant at the core base and decrease further upcore (Figure 4.12). In addition to pelagic species, there is a diverse assemblage of plant- and mud-associated species including *Alonella exigua*, *Alonella nana*, *Eurycercus lamellatus*, *Graptoleberis testudinae*, *Alona affinis* and *Leydigia leydigi*. Further, *Pleuroxus truncatus*, *Chydorus piger* and *Alona rustica* are present in low numbers exclusively in this zone (Figure 4.11).

This zone is not present in core CUNS1 (Figures 4.13, 4.14) and therefore macrofossil data only of CUNS2 are used. Abundant plant macrofossils in this zone in CUNS2 include seed fragments of *Nymphaea alba*, seeds of *Juncus*, and uncalcified oospores of *Chara hispida* agg., *Chara contraria* agg. and *Nitella* cf. *flexilis* (Figure 4.15). Oblong *Plumatella* forms are also abundant, not recorded upcore of the zone with the exception of levels 42.5 and 48.5 cm (zone 1b), whereas molluscs are markedly absent with the exception of the Sphaeriidae (Figure 4.16).

Zone 1b

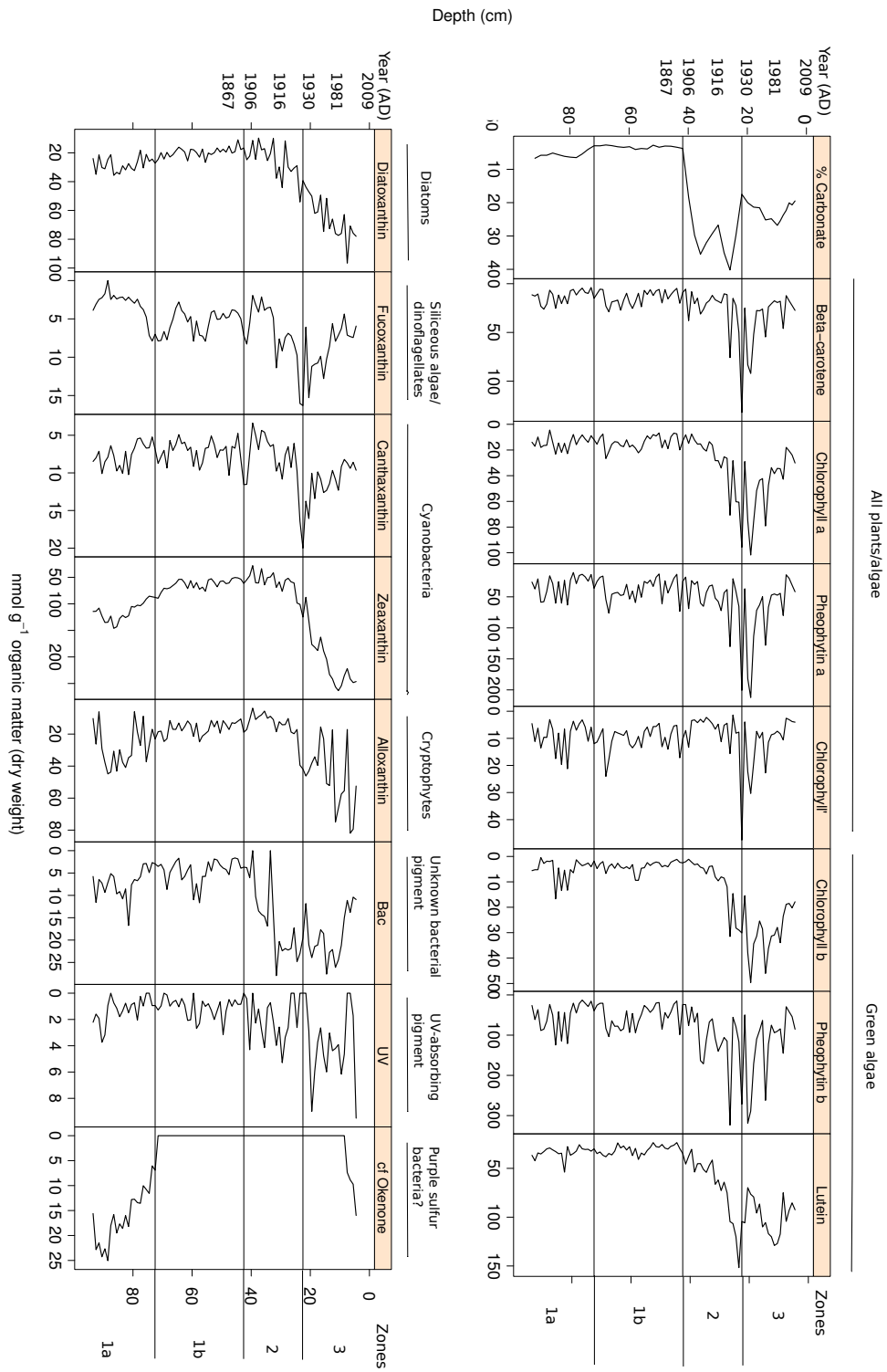


Figure 4.9: CUNSS2 pigment data taxonomically ordered.

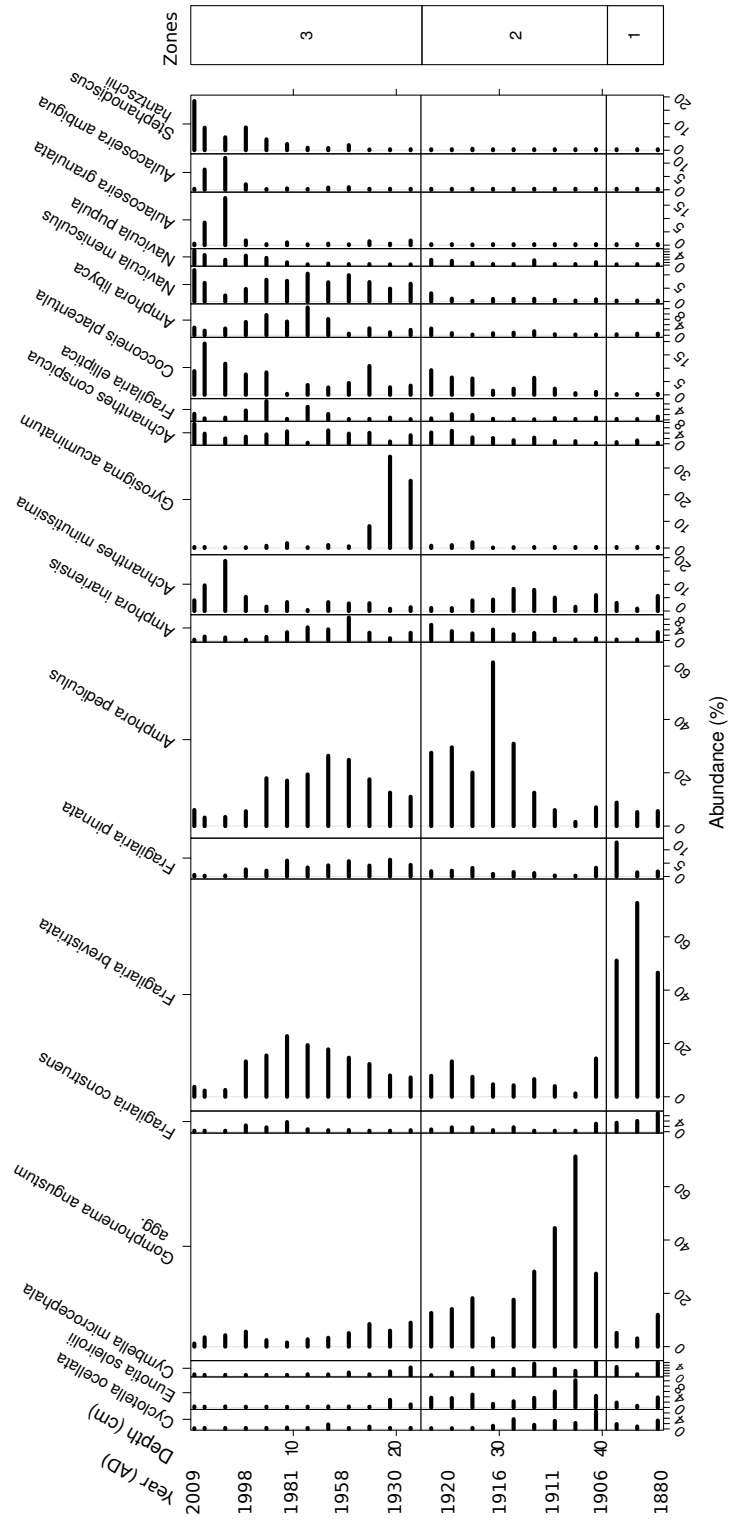


Figure 4.10: CUN2 diatom % abundance data, showing only taxa appearing in more than 5 core levels with a maximum abundance of at least 5%
Data from Stone (2012).

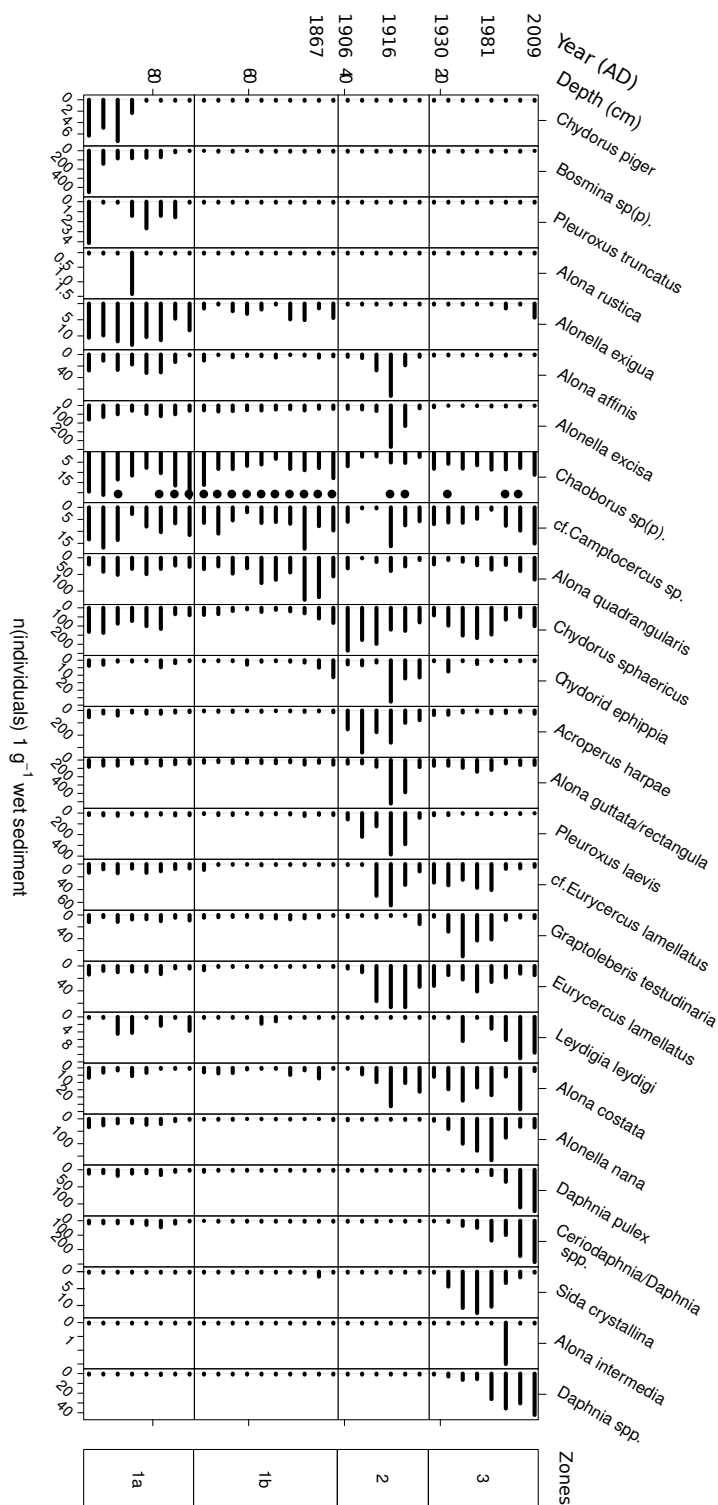


Figure 4.11: CUNSS2 subfossil cladoceran absolute abundance data. Filled circles in the column for *Chaoborus* indicate presence of *Chaoborus obscuripes/crystallinus*.

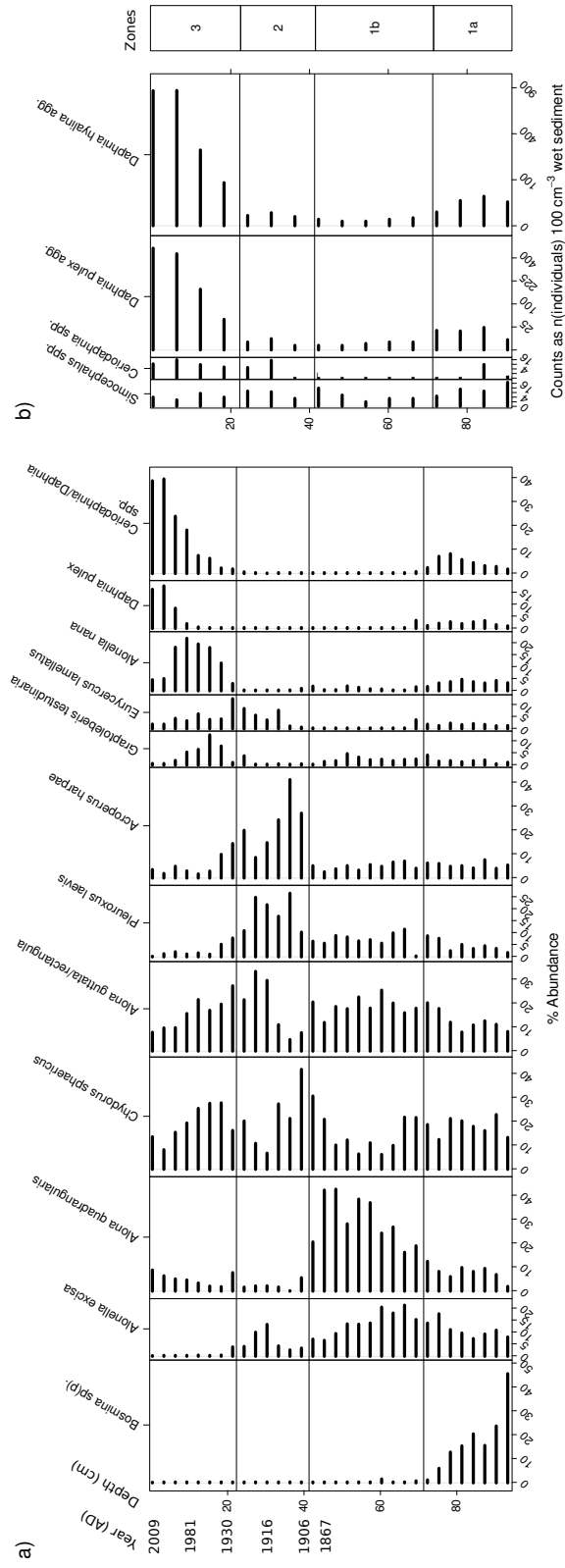


Figure 4.12: CUN2: a) subfossil cladoceran % abundance data, showing only taxa appearing in more than 3 core levels with a maximum abundance of at least 7%. b) Counts of ephippia from macrofossil analysis.

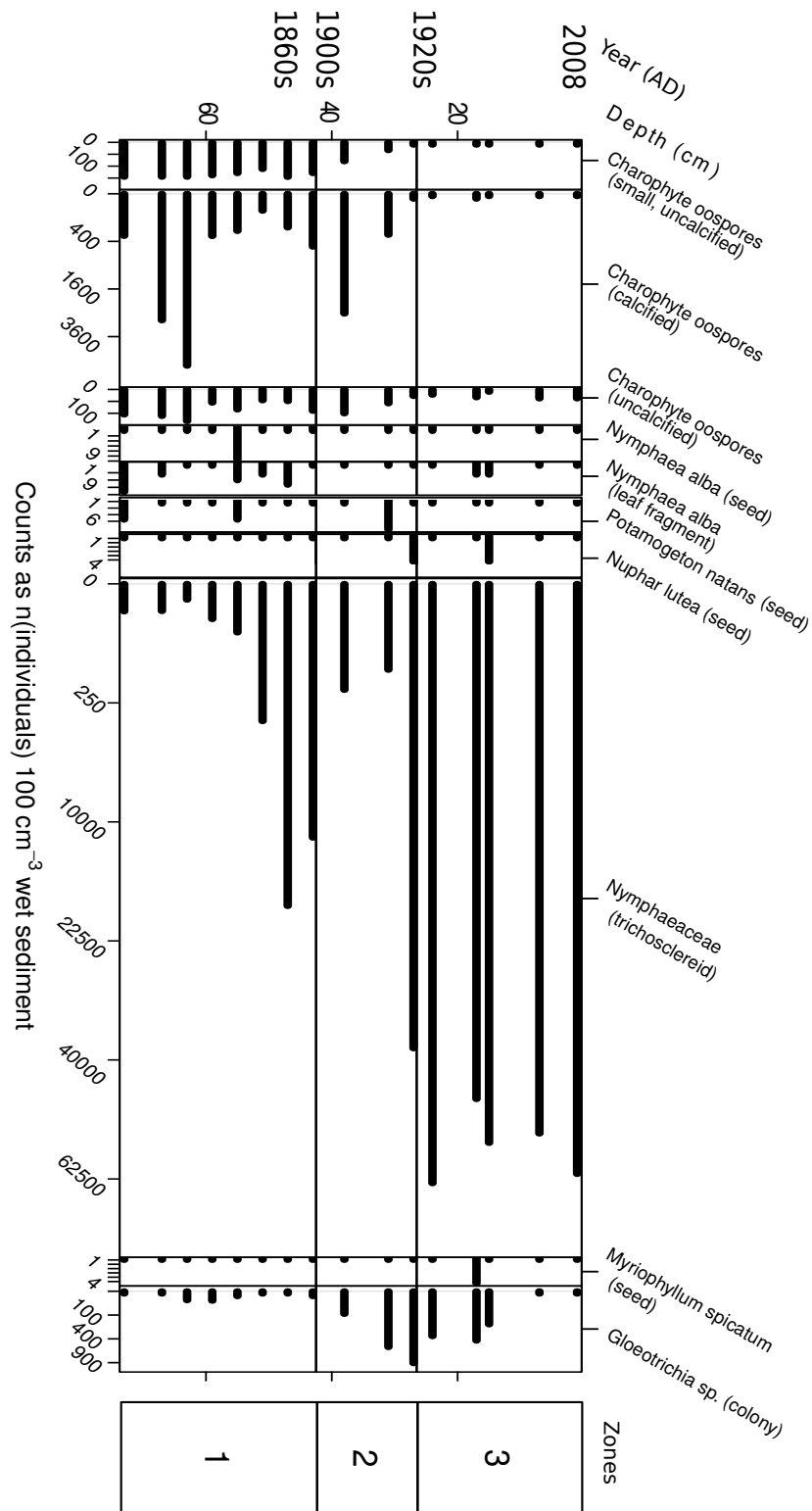


Figure 4.13: CUNSI plant macrofossil data expressed on square-root scale. The zone lines represent the carbonate zones of CUNSI.

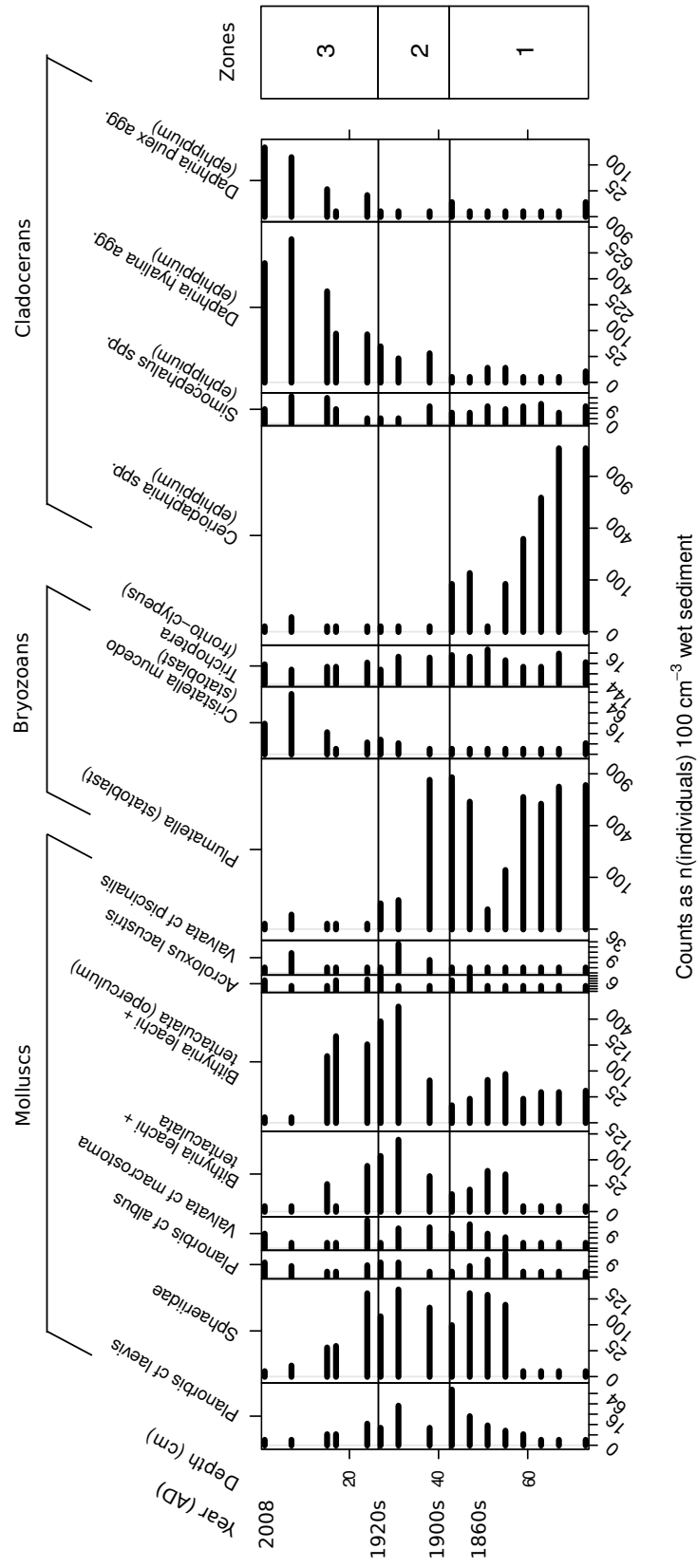


Figure 4.14: CUN51 animal/bryozoan macrofossil data expressed on square-root scale. The zone lines represent the carbonate zones of CUN51.

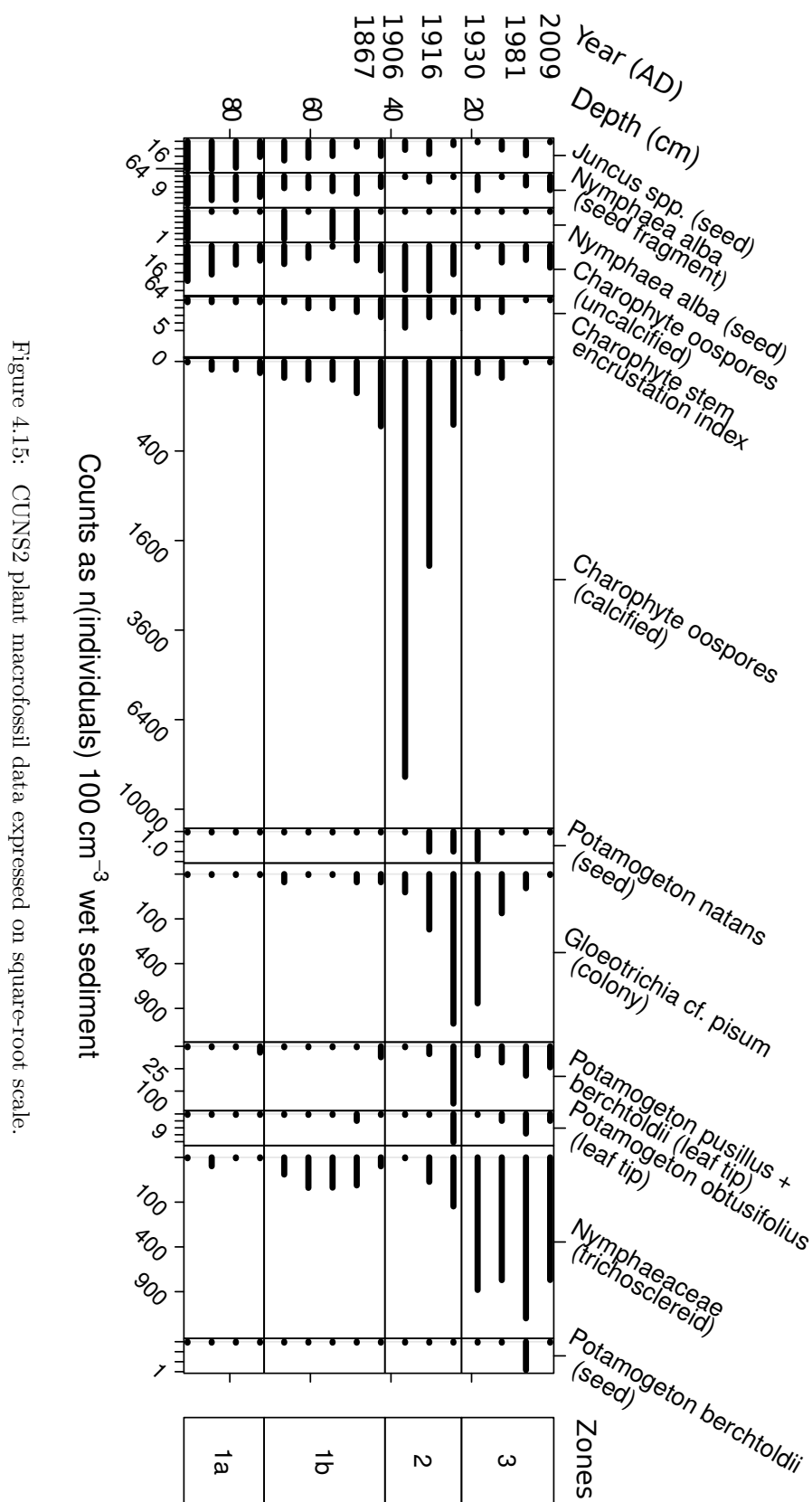


Figure 4.15: CUNSS2 plant macrofossil data expressed on square-root scale.

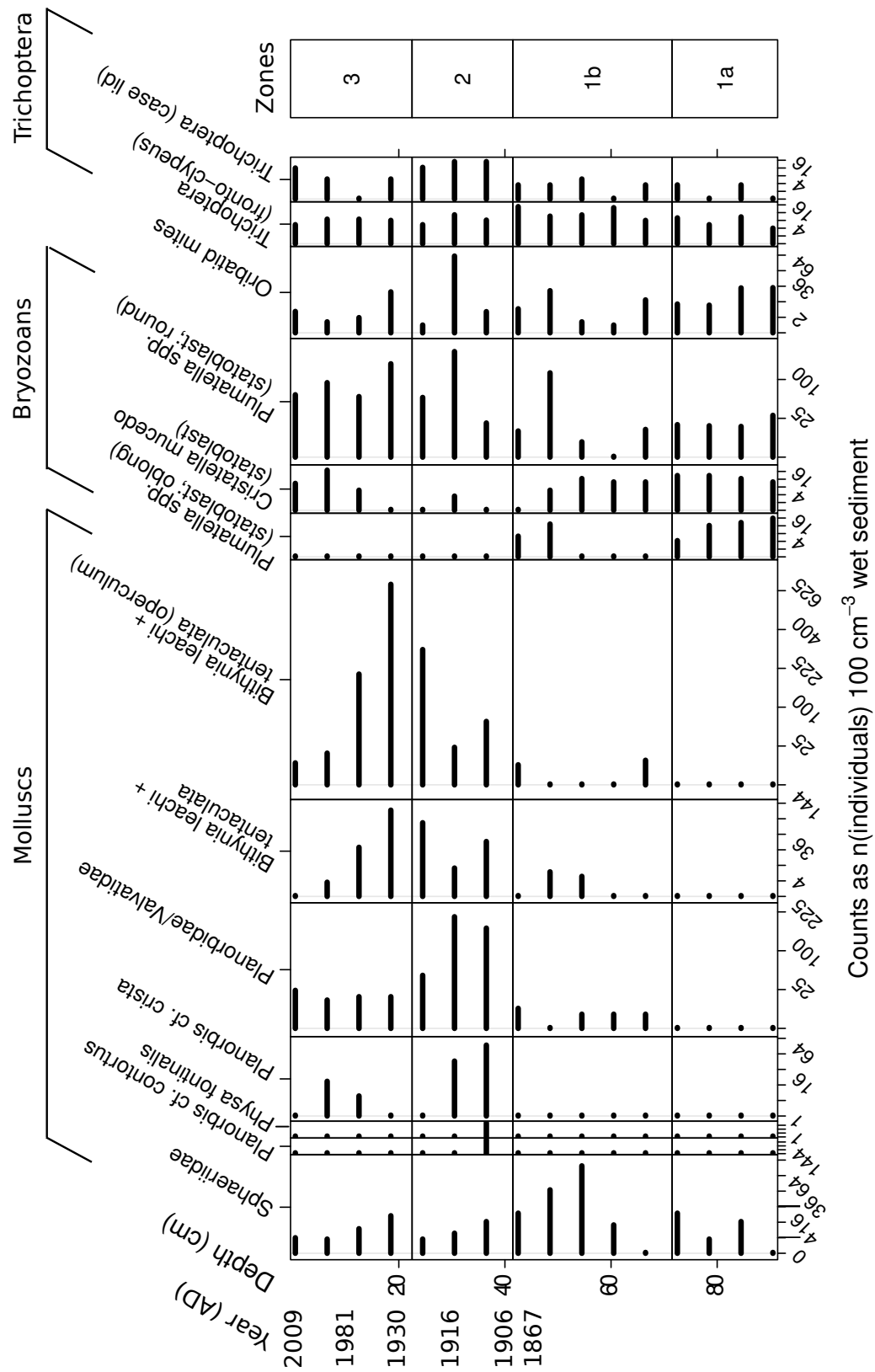


Figure 4.16: CUN2 animal/bryozoan macrofossil data expressed on square-root scale.

The concentrations of zeaxanthin, diatoxanthin and alloxanthin in CUNS2 are low in zone 1b compared with 1a, and no cf. okenone is recorded (Figure 4.9). The decrease in zeaxanthin is reflected in an increase in the ratio of chlorophytes to cyanobacteria. However, overall productivity as indicated by generic pigments is largely unchanged, and pigment concentrations fluctuate without showing clear upcore trends. Diatoms in CUNS2 are recorded only for the uppermost 4 cm of the zone by three samples (level 41.5 is appended to this zone rather than zone 2) (Figure 4.10). The community is dominated by benthic *Fragilaria* such as *Fragilaria brevistriata*, and to a lesser degree peri/epiphytic taxa such as *Gomphonema angustum* agg. and *Amphora pediculus*. The assemblage consists largely of nutrient-intolerant *Cyclotella* spp. and *Fragilaria* spp. characteristic of pre-eutrophication conditions of high light penetration (Moss, 1979; Padisak and Reynolds, 1998; Meriläinen et al., 2000; Bennion et al., 2001; Cremer and Wagner, 2003; Gregory-Eaves et al., 2003; Kulikovskiy et al., 2010; Bennion et al., 2011).

In contrast to zone 1a, cladoceran diversity and overall abundance in CUNS2 is low in zone 1b (Figure 4.11). The community is dominated by the plant-associated chydorids *Chydorus sphaericus*, *Alona guttata/rectangula* and *Alona quadrangularis*, the latter increasing in abundance upcore (Figure 4.12). In CUNS1, macrofossils of small *Ceriodaphnia* spp. ehippia are abundant at the core base (>1000), decreasing upcore (<100 above 60.5 cm) (Figure 4.14). While this cannot be directly compared to the subfossil cladoceran data of CUNS2, maximum abundance would correspond either to the equivalent of the upper end of zone 1a or the lower end of zone 1b.

In CUNS2, plant macrofossils in zone 1b are characterised by lesser abundance of *Nymphaea alba* seeds, *Juncus* seeds, and charophyte oospores compared with zone 1a (Figure 4.15). However, in CUNS1, high concentrations of calcified oospores are present throughout zone 1 (Figure 4.13), also associated with higher sediment carbonate concentrations (Figure 4.5). These oospores are distinctly smaller than those in CUNS2, suggesting dominance in the shallower zone of a different species, identified by morphotype as *Chara aspera*. While *N. alba* seed fragments and seeds are present throughout zone 1b, no *N. lutea* remains were found, indicating *N. alba* may have been more numerous in this period. Trichosclereids in both CUNS1 and CUNS2 show a small peak in the zone although the synchrony between the two is difficult to ascertain (Figures 4.13, 4.15). *Potamogeton* seeds in this zone were

found in CUNS1 only, with *Potamogeton natans* seeds scattered throughout the zone, and one *Potamogeton praelongus* seed at a depth of 70.5 cm.

Mollusc remains, of relatively low abundance lower in the zone, increase upcore in both cores and become abundant near the zone 1/2 boundary (Figures 4.14, 4.16). In CUNS2 this is mainly owing to the Sphaeriidae, reaching a maximum of 50-100 individuals 100 cm⁻³ at 54.5 and 48.5 cm, whereas in CUNS1 several different taxa become more abundant (*Bithynia leachi/tentaculata*, *Planorbis* cf. *albus*, Sphaeriidae, *Valvata* cf. *macrostoma*).

Zone 2 (beginning of the 20th century - early 1920s); 42.5 - 26.5 cm (CUNS1); 42.5 - 22.5 cm (CUNS2) In CUNS2, concentrations of most algal/bacterial pigments begin to change in zone 2 (Figure 4.9). Chlorophyte pigments increase in this zone, first indicated by pheophytin *b* and lutein with a slight lag in chlorophyll *b*. These pigments are paralleled by an increase in chlorophyll *a*, suggesting that chlorophyll *a* concentrations are driven by chlorophytes. The unidentified bacterial pigment shows the most dramatic response within its own concentration range, increasing upcore from the base of the zone. The ratio of chlorophyte pigments to cyanobacterial pigments shows peaks matching those of carbonate, at 36.5 (1910) and 26.5 cm (1919), suggesting chlorophytes were particularly abundant during the time period covered by maximal marl precipitation. Cyanobacterial pigments stay within background variability suggesting no major increase in cyanobacteria during this period. Similarly, diatoxanthin and fucoxanthin remain largely within previous concentration limits, although fucoxanthin increases slightly upcore. Total productivity increases towards the top of the zone as evidenced by increases in sediment pigment concentrations.

The ratios of chlorophyll *a* and pheophytin *a* to the oxidative degradation pigment chlorophyll *a'* increase at 40.5 cm, immediately above the zone 1/2 boundary at 42.5 cm (1904), and remain high and variable throughout the zone. The proportional decrease in chlorophyll *a'* indicates less oxic sediment conditions throughout the zone (Figure 4.8).

Diatoms in CUNS2 change markedly between zones 1 and 2 (Figure 4.10). The relative abundance of epiphytic species increases from values around 40% to values around 70% with a reciprocal decline in benthic species (Figure 4.17). The epiphytic community is dominated by *Gomphonema angustum* agg. and *Amphora pediculus* agg, with persistence of the relatively nutrient-sensitive *Cymbella microcephala* (Schneider et al., 2000). Upcore from 31.5 cm

(1916) there is a slight shift towards the more nutrient-tolerant *A. pediculus* (Kwandrans et al., 1998; Garcia-Rodriguez et al., 2007; Csilla Stenger-Kovács et al., 2007) relative to the more nutrient-intolerant *Gomphonema pumilum*, *G. angustum* agg. and *Cyclotella ocellata* (Brüchmann and Negendank, 2004; Csilla Stenger-Kovács et al., 2007; Veraart et al., 2008; Urrea-Clos, 2010). Diatom abundance is particularly high at 29.5 cm, attributable mainly to an increase in epiphytes (Figure 4.17).

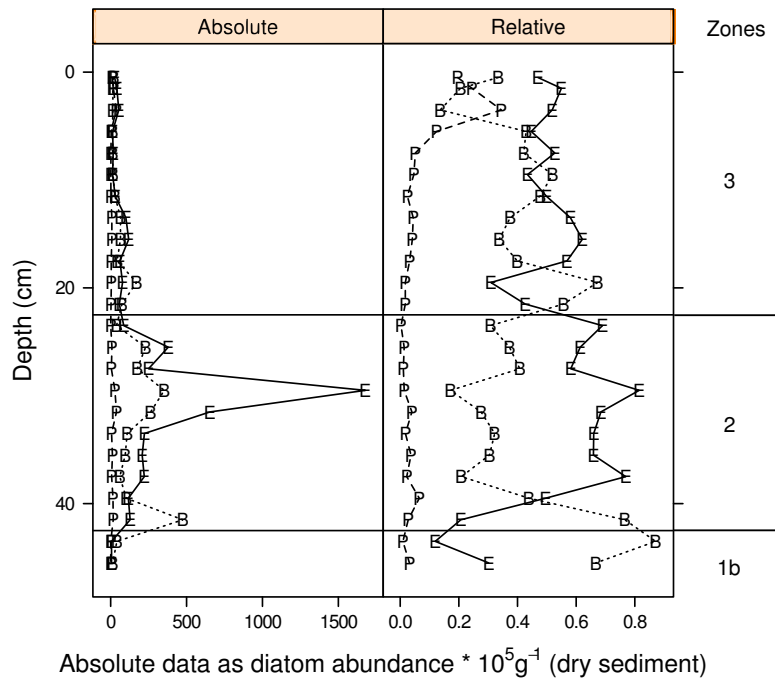


Figure 4.17: CUNS2 diatom data as absolute and relative abundance according to habitat. “B”, “E”, “P” refer to benthic, epiphytic, and pelagic, respectively. Data from Stone (2012)

There is a relatively abrupt change in the plant-associated chydorid community at the base of zone 2 in CUNS2. *P. laevis* and *A. harpae* increase and become dominant (Figure 4.12), followed by an increase in *E. lamellatus*, *A. excisa*, *A. guttata/rectangula* and *A. affinis* (Figure 4.11). *A. costata* increases gradually throughout the zone, while *A. quadrangularis* decreases in abundance and *A. exigua* disappears from the record. Total cladoceran abundance is at a maximum at 30.5 cm (1916) and declines to less than a third within 6 cm, representing a time period of approximately 15 yrs. This decrease is related to declines in most species, and total abundance at the upper end of zone 2 is similar to zone 1. *Ceriodaphnia* ephippia in CUNS2 in part mirror this pattern, with relatively high numbers at 30.5 cm, although no

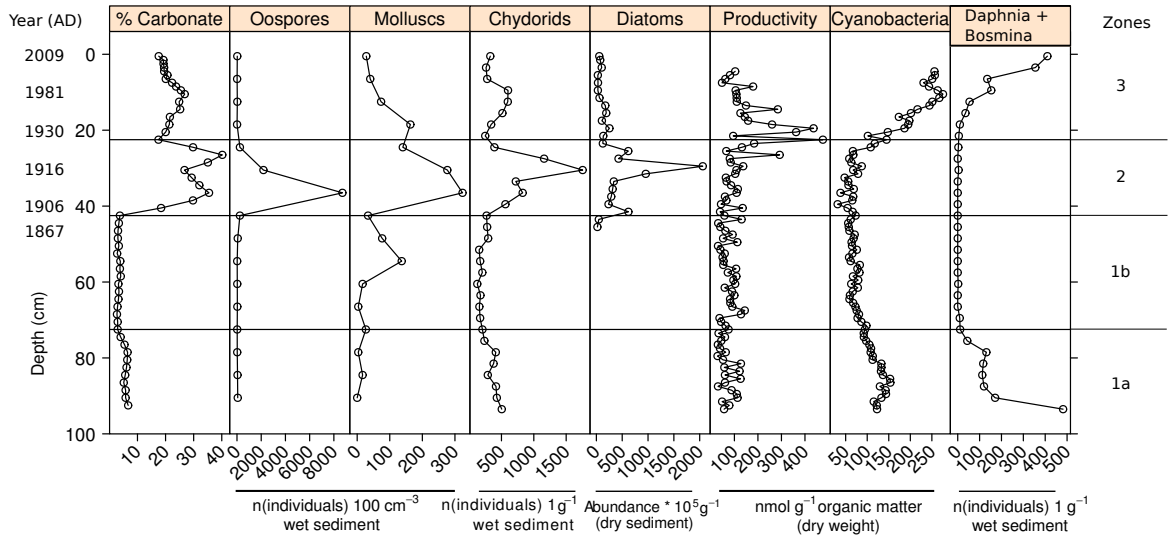


Figure 4.18: Abundances of selected organisms (concentrations as in individual diagrams), with “Productivity” referring to total productivity as given by certain pigments (see Methods).

substantial decline in their abundance occurs upcore (Figure 4.12). In contrast, in CUNS1, *Ceriodaphnia* ehippia are most abundant in zone 1 (Figure 4.14), however, these ehippia are very small and probably represent a different species. Pelagic cladoceran remains are absent or rare in both cores in zone 2.

Two macrophyte phases can be identified in the cores, associated with the lower end of the zone and the first carbonate peak, and the upper end of the zone and the second carbonate peak. Oospores in both cores reach high abundances at the lower end of the zone (maximally 2698 [CUNS1, 38.5 cm] and 8707 [CUNS2, 36.5 cm] 100 cm^{-3} ; early 1900s), paralleled by high abundances of encrusted stem remains (Figures 4.13, 4.15). The presence of several oospore morphotypes suggests a community consisting of several species. A maximum of six and four morphotypes were found within a given sample in CUNS1 and CUNS2, respectively, belonging to *C. aspera*, *C. hispida* agg. and/or identified as *Chara* cf. *vulgaris* and *Chara* cf. *globularis*. These aggregates include both shallow- and deep-growing species.

In the top section of this zone in both cores, containing the upper carbonate peak, oospores decrease in abundance and conversely, colonies of *Gloeotrichia* cf. *pisum* and Nymphaeaceae trichosclereids increase in number, the former dramatically (approximately threefold in both cores). In this section of CUNS2, leaf and seed remains of *Potamogeton obtusifolius*, *Potamogeton pusillus/berchtoldii* and *Potamogeton natans* also increase (Figure 4.15). In CUNS1,

one *Nuphar lutea* seed in the upper end of zone 2 (27.5 cm) confirms the presence of *N. lutea* in addition to *N. alba* in the lake from the early 1920s.

Of the animal remains in CUNS2, those of molluscs in particular attain highest abundances in the lower section of this zone (Figure 4.18), also corresponding to the highest morphotype diversity (Figure 4.16) at the 36.5 cm carbonate peak. In both cores, *Bithynia leachi/tentaculata* remains increase towards the top of the zone (Figures 4.14, 4.16).

Zone 3 (late 1920s - 2008/2009); 26.5 cm/22.5 cm to core top (CUNS1/CUNS2)

Over the zone 2/3 boundary in CUNS2, following the decline in carbonate concentration, generic pigments as well as chlorophyte pigments increase and attain maxima at around 20 cm (1920s) (Figures 4.8, 4.9). However, they decline steeply thereafter. Fucoxanthin and canthaxanthin show a similar pattern of increase and decline, while diatoxanthin, zeaxanthin and alloxanthin continue to increase throughout the zone. On average, concentrations of the aforementioned pigments are higher in zone 3 than -2. Concentrations of the unidentified bacterial pigment remain relatively unchanged in this zone, but decrease in the uppermost 10 cm (1980s to 2009). Further, in the top 10 cm, cf. okenone reappears in the record.

Pelagic diatoms in CUNS2 increase in abundance throughout zone 3, including species associated with eutrophic conditions such as *Aulacoseira* spp. and *Stephanodiscus hantzschii* (Brugam, 1983; Reynolds, 1984; van Donk and Kilham, 1990; Reynolds et al., 2002; Negro and de Hoyos, 2005) (Figure 4.10). Maximal abundance of pelagic taxa is reached within the top 5 cm, where the proportion of benthic taxa decreases (Figure 4.17).

The remains of pelagic cladocerans in the macrofossil record of both cores, and the cladoceran record of CUNS2, increase in this zone, represented by *Daphnia* spines/ephippia and *Ceriodaphnia* ephippia (Figures 4.11, 4.12, 4.14). Absent in the record since the time period of zone 1, two *Bosmina* spp. individuals were found in the uppermost sample of the core. In the CUNS2 cladoceran record, species abundant in the preceding zone decline with the exception of *C. sphaericus* which increases in concentration (Figure 4.11). Species such as *S. crystallina*, *A. nana*, *L. leydigi* and *G. testudinaria* also increase, while the abundance of *A. costata* fluctuates around the abundance attained in zone 2. The uppermost 5 cm are marked by maximum *Daphnia* abundance, and a decrease in chydorids except for *A. exigua* (reappears in the record) and *A. quadrangularis* (increases slightly) (Figure 4.11). Total cladoceran

abundance increases again, but fluctuates and is at the core top driven mainly by the increase in pelagic species (Figure 4.18). Contrary to the pattern in CUNS2, *Simocephalus ephippia* in CUNS1 increase towards the core top (Figures 4.12, 4.14).

The patterns of plant macrofossils of CUNS2 and CUNS1 are similar in this zone. *Potamogeton natans* seeds disappear from the record at the base of the zone, and Nymphaeaceae trichosclereids increase dramatically. In CUNS2 fine-leaved *Potamogeton* leaf remains persist, and a *Potamogeton pusillus/berchtoldii* seed was found in the top 10 cm. In CUNS1, a single *Nuphar lutea* seed was found at 15.5 cm (1960s), and a single seed of *Myriophyllum spicatum* at 17.5 cm (1950s). This is the only fossil record of *Myriophyllum spicatum* in the lake.

In both CUNS1 and CUNS2 animal fossils show two major changes. Mollusc remains decrease markedly in abundance in the upper half of the zone (Figures 4.18, 4.14), whereas *Cristatella mucedo* remains increase (Figures 4.16, 4.14).

4.4.4 Community-level change; comparisons between proxies and relationship with core carbonate content in CUNS2

Stratigraphic clusters and ecological distances Cluster analyses on CUNS2 data revealed fairly similar stratigraphic divisions for subfossil cladocerans and macrofossils (plant and animal excluding cladocerans). Pigments exhibited a slightly different pattern, which may be due to a range of factors. For example, fossil pigments do not represent species or genus-level detail, which may mask major changes in species composition. It also follows that individual pigment concentrations can fluctuate markedly throughout core profiles owing to differences in pigment composition between species rather than abundance per se, potentially resulting in somewhat erratic groupings. For simplicity, only ordination on grouped pigment data is displayed. In general, all the above proxies fall into four major sections within two parent clusters. The boundaries of the sections correspond relatively well to the carbonate zones 1a,b; 2; 3 (72.5, 42.5, 22.5 cm).

Roughly, the sections represent the core base, lower middle, upper middle, and top (Figures 4.19 and 4.20). For cladocerans, the core base and top sections fall within one cluster, and the two mid-core sections into the other. In contrast, macrofossil and pigment data divide into core base and core top clusters. Therefore, the similarity between the cladoceran community composition in the core top and base sections is not reflected by macrofossil and

pigment clusters where the most recent communities are distinct from historical communities.

The base section boundary for macrofossils and pigments is at 72.5 cm, and for cladocerans, 69.5, which is in good agreement with carbonate zones 1a and 1b. Cladoceran (Figure 4.19) and pigment data (not shown) display distinct change in NMDS ordination space at the boundary, as distances between points in this region increase (Figure 4.19). Correspondingly, core levels within these zones are more densely clustered. Macrofossil data display relatively more change further upcore in zone 1b, between 66.5 and 60.5 cm, and 54.5 and 48.5 cm (Figure 4.19).

Biological community change is also in agreement with the boundary between zones 1 and 2. The sections of macrofossils and cladocerans indicated by cluster analysis terminate at 42.5 cm, corresponding to the beginning of carbonate zone 2. Although the corresponding pigment section terminates at 39.5 cm, in close agreement with the other proxies, most samples below a core depth of 54.5 cm belong to the section further upcore, showing similarities across time segments. This seesaw pattern is reflected in ordination space as close proximity between these two sections (Figure 4.20). In ordination space, the most distinct community change of subfossil cladocerans and pigments occurs between 36.5 and 42.5 cm, and for macrofossils, 30.5 and 42.5 cm (Figures 4.19 and 4.20), which encompasses the area of fluctuation in carbonate content.

The lower boundaries of the upper core sections are slightly different between proxies and do not correspond exactly to the carbonate zone 2 and 3 boundary at 22.5 cm. For cladocerans, the boundary is 18.5 cm, and for macrofossils and pigments, 24.5 cm (Figure 4.19). Therefore, a change in cladoceran communities precedes the end of carbonate zone 2, and change in pigments and macrofossils are delayed and overlap with zone 3. For macrofossil data, this may in part be caused by the lower resolution of the data set. Further, the division between zones 2 and 3 in ordination space is less obvious compared to the other zones, as community change in all proxies is relatively even/constant through this section of the core. In ordination space, the uppermost samples of both pigments and cladocerans are more similar to the core base samples than those lying in zones 2 and 1b. This is in contrast to macrofossil data. The similarity between the core base and top of grouped pigment data is largely due to the presence of *cf. okenone* and relatively high abundance of cyanobacterial pigments, and for cladocerans, due to abundances of pelagic cladocerans and a few chydorid species.

The PCA on grouped pigment data shows that core levels within the region of high carbonate

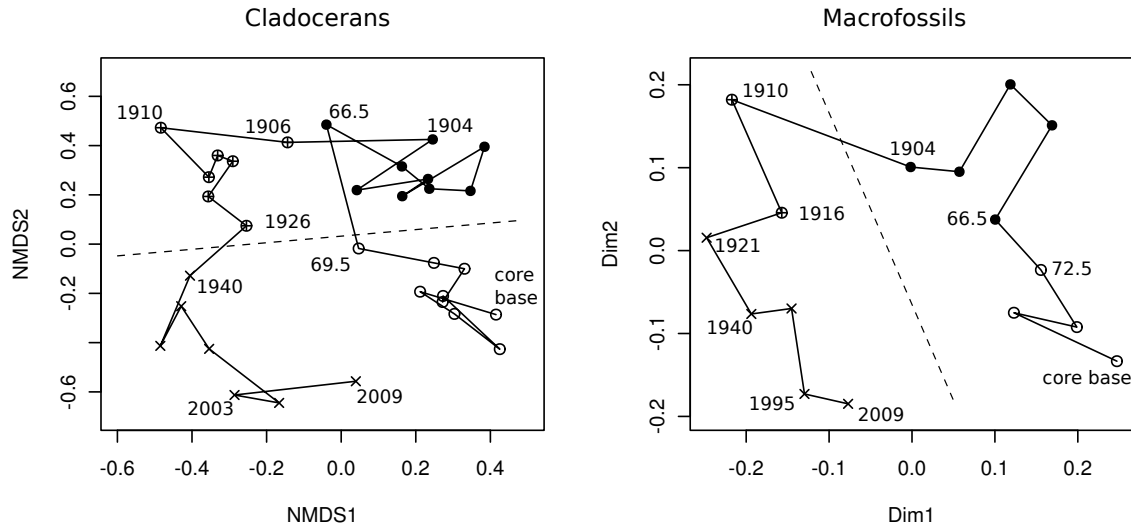


Figure 4.19: NMDS on CUN2 cladoceran and macrofossil data, respectively; x and y axes of cladoceran data were reversed to mirror the arrangement of the macrofossil data. Plotting symbols denote assemblages indicated by cluster analysis, and the dotted line shows the division between the two parent clusters. Core levels, and where available, dates as years, are shown between the core base and top where larger community shifts take place

content are generally not characterised by cyanobacterial or cryptophyte pigments, which is more characteristic of the core top and base levels. The trend in total productivity is nearly completely superposed on that of chlorophyte pigments, indicating chlorophytes are the dominant algal group over time, with maxima in the region of declining carbonate content. The uppermost 10 cm are indicative of lower total productivity.

Change as indicated by PCs of the cladoceran, diatom and macrofossil data show similar patterns to the core carbonate profile (Figure 4.21). Pigments were analysed both individually and as taxonomic groups (chlorophytes, cyanobacteria, diatoms, etc.). PCs explained 64% (diatoms), 76% (individual pigments), 87% (grouped pigments), 75% (cladocerans), and 90% (macrofossils) of the variance in the data sets. However, these values should not be given too much weight as there is no value in explaining noise, and in this respect 100% variance explained would imply the PC has been overfitted (see e.g. Økland, 1999; Oksanen et al., 2011).

Given the difference in the resolution of the data sets, the match between community change and carbonate content is striking. All communities are relatively stable within zone 1a show-

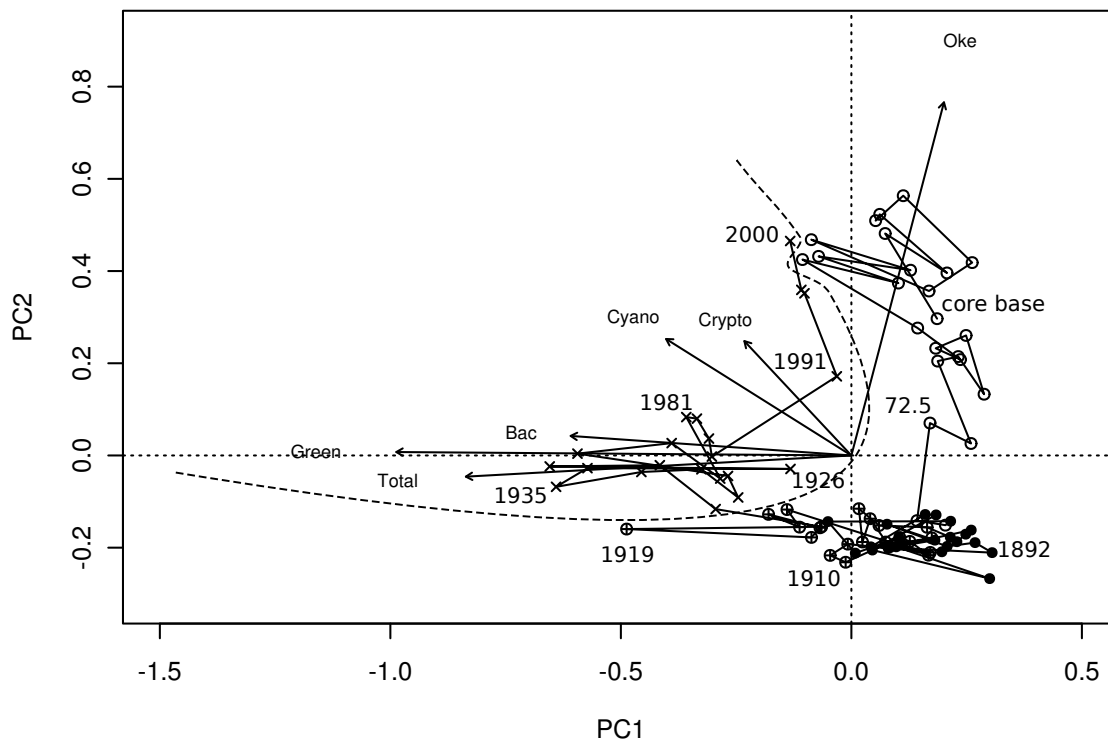


Figure 4.20: PCA on CUN2 pigment data as taxonomic groups. Separate clusters as per cluster analysis are indicated by different symbols. The core top samples are in a separate cluster, indicated by the dashed arc, and the rest nest within a second group, with mid-core levels as a dense cloud in ordination space. The uppermost core levels plot close to the core base levels due to the reappearance of cf. okenone in the record.

ing no marked change with time, with the exception of grouped pigments. With the exception of macrofossils, all proxies show change at the zone 1a -b boundary where core carbonate content decreases slightly. While individual pigments become more variable compared with zone 1a, grouped pigments change to the same degree as previously. For cladocerans, community change is slightly upcore of the zone boundary, as also indicated by cluster analysis grouping level 69.5 with zone 1a. On the whole, zone 1b is characterised by stability in macrofossils and cladocerans, and change of constant magnitude in pigments.

The most dramatic community change is associated with the zone 1/2 boundary where core carbonate content increases. This change is also captured in the diatom data where two samples within zone 2 were analysed. The third sample, from 41.5 cm, is stratigraphically unmatched by any other proxy and therefore the “delay” in response may simply be an artefact

of differing resolution in data sets. This sample is therefore appended to zone 1b for purposes of ecological interpretation, and the marked change between 41.5 and 39.5 cm is regarded as a response tied with the increase in core carbonate. This boundary is also marked by changes in pigments (individual and grouped), cladoceran and macrofossil communities. Within zone 2, there are marked differences in communities related to the lower (36.5 cm) and the upper (26.5 cm) carbonate peaks. The lower section is associated with large variability in diatoms and pigments, and one step-like change in macrofossils. In contrast, beyond the change at the zone boundary, cladoceran communities show no marked change within this section (slight community change is apparent in absolute cladoceran data; Figure 4.11). The upper section corresponds to very little change in diatoms and cladocerans, and pigment data are less variable in this section compared with the lower section. Macrofossils change markedly.

The boundary between zones 2 and 3 is mirrored by change in the PCs of cladocerans and macrofossils, and also by a slight change in diatoms (while only slight, this corresponds to an increase in benthic, and a reciprocal decrease in epiphytic, taxa). In zone 3, pigments become less variable and show slight, constant change upcore. Diatoms and cladocerans also show less change in this zone, while macrofossils continue to change to a depth of 12.5 cm above which they become less variable. In contrast, the uppermost 10 cm of the core marks increased change in cladocerans and diatoms.

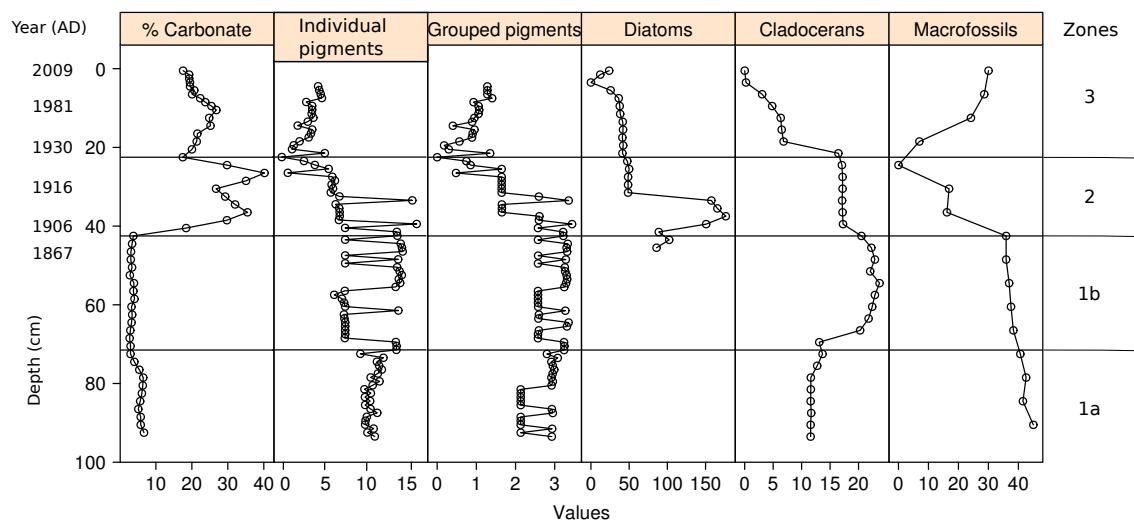


Figure 4.21: PCs of macrofossil, cladoceran, pigment and diatom data of CUN2 together with the CUN2 core carbonate profile. The values on the x-axis, and the direction of the PCs on the x-axis, are irrelevant for interpretation.

Constrained analyses of subfossil cladoceran data from CUNSW2 CCA with Ca, P and Fe on CUNSW2 cladoceran data using WA and LC scores produced similar results, suggesting that all three elements are suitable for describing cladoceran community change (data not shown). Because PCA was chosen to better represent the data in earlier analyses, interpretation is based on RDA (Figure 4.22), explaining 53.4% of the variance. To assess the linearity of gradients, concentration contours of Ca, P and Fe were drawn alongside site scores of NMDS performed on Hellinger-transformed data using Euclidean distances to mimic the projection performed by RDA. All three elements are significant to the 0.001 level, although temporal autocorrelation was not accounted for. Contours reveal relatively linear change along gradient arrows, with the exception of Fe for levels within zone 1 (Figure 4.22; Ca and P only). Thus, levels close to the core base do not necessarily plot accurately into linear Fe space - the relationship is most accurate for the uppermost samples with low Fe concentrations. Poor projection was in part expected, as Fe concentrations do not change markedly below 15.5 cm (Figure 4.6).

While core levels between the core top and 18.5 cm (zone 3) are associated with high P concentrations, variation in core levels associated with higher carbonate concentrations (zone 2; 21.5 - 39.5 cm) are less related to P, and described better by Ca as shown by the perpendicular relationship between Ca and P in this section and the variation in samples predominantly along the Ca gradient (Figure 4.22). In general, zones 3 and 2 encompass the core levels of most variability along the element gradients, whilst core levels within subzones 1 a and -b cluster more tightly within a smaller range of element concentrations as shown through RDA. In NMDS space, however, there is community change within zone 1 subzones and this change is therefore related to factors other than those associated with change further upcore.

4.5 Discussion

4.5.1 Drivers of ecological change in Cunswick Tarn

Selected biological and chemical proxies were used to define premises for the interpretation of the ecological history of Cunswick Tarn, and therefore certain assumptions have been made.

Firstly, Cunswick Tarn has probably been fishless over the period represented by the sediment record of CUNSW2. This is testified by (i) a lack of fish scales and fish leech cocoons (*Piscicola geometra*), the latter commonly found in sediment cores from shallow lakes contain-

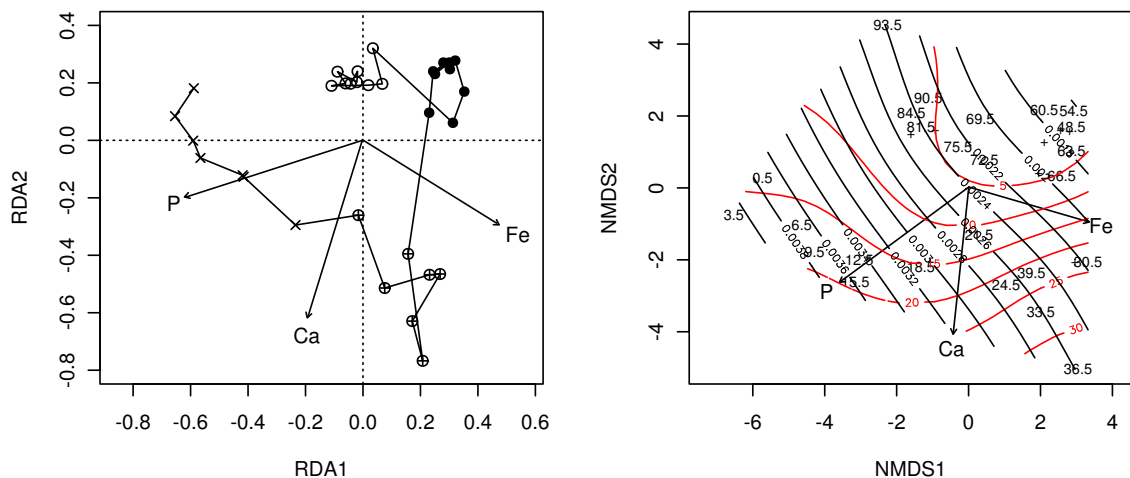


Figure 4.22: CUNSW2 cladoceran and elemental ordinations. Left: RDA showing the location of sites under constraint of Ca, P and Fe. Stratigraphic zones are denoted as in Figure 4.19. Right: Unconstrained NMDS on the same data showing concentration contours of P (black) and Ca (red) and gradient arrows produced by vector fitting. Contours of Fe are omitted, and sites that would plot with overlapping labels are shown as “+”, to avoid congestion.

ing fish (Odgaard and Rasmussen, 2001); (ii) the presence of large-bodied cladoceran species throughout the core; (iii) *Chaoborus obscuripes/crystallinus* larval mandibles, found occasionally throughout the core (Figure 4.11). These species are non-migratory and are usually associated with fishless lakes (Luoto and Nevalainen, 2009; Tolonen et al., 2012; Palm et al., 2012). Therefore, it is assumed that the food web has been devoid of fish. Consequently, all biological changes in the sediment cores are attributed to changes in nutrient loading, food availability, and habitat structure.

Secondly, the designation of key ecological phases in Cunswick Tarn is based on changes in the concentrations of sedimentary P, carbonates and pigments. Using P as a eutrophication proxy can be controversial due to redox effects and upcore mobility (Carignan and Flett, 1981; Engstrom and Wright, 1984). For example, Anderson et al. (1993) compared diatom-inferred P with monitored in-lake TP and sedimentary P in two Irish lakes and interpreted sedimentary P as a response to sediment redox conditions rather than a direct proxy for eutrophication. However, overall sediment core P concentrations have often been used as a proxy for eutrophication when such effects are accounted for (Pennington, 1981; Rees et al., 1991; Brezonik and Engstrom, 1998; Neumann et al., 2002; Hobbs et al., 2005).

In Cunswick Tarn, core P concentrations are not positively correlated to Mn concentrations, and the highest P concentrations plot at intermediate Fe:Mn - low [Fe] regions (Figure 4.7). Such patterns are inconsistent with redox-mediated P release, suggesting that redox state is not driving the pattern of P concentrations in the core (Mackereth, 1966; Hobbs et al., 2005). Finally, the mobile section of P in sediment cores tends to lie within the uppermost 5 - 15 cm (Carignan and Flett, 1981), which is associated with P release through internal loading (Søndergaard et al., 2001). Mobility at these depths does not necessarily interfere with the general downcore pattern of P apart from the very top (Søndergaard et al., 1999). As P concentrations in Cunswick Tarn begin to increase at a depth of 40 cm, P mobility can be considered unlikely to be an explanatory factor. Consequently, the pattern of increasing upcore concentrations is interpreted as a eutrophication signal.

Increased productivity is also associated with increases in sediment carbonate concentrations, since biologically induced carbonate precipitation is inherently dependent on photosynthesis (Schelske and Hodell, 1991; Anderson et al., 1993; Teranes et al., 1999; Neumann et al., 2002; Robertson et al., 2007). Initial increases in core carbonate and P upcore of 42.5 cm (early 1900s) are in agreement with eutrophication. Coincident with these changes, CUNS2 carbonate stable isotope data (Chapter 5) indicate hydrological changes involving less closed conditions. Given increased throughflow and therefore lower lake water levels, the dramatic increase in core carbonate content could in part be argued to result from a shift from a deep-water to a littoral core signal (e.g. Bradshaw et al., 2005)). This is, however, considered unlikely because (i) the carbonate increase is also seen in core CUNS1 which contains abundant charophyte remains and carbonate in deeper core levels; (ii) CUNS1 was recovered at a water depth 1.5 m shallower than CUNS2 and would thus be expected to function as a palaeolimnological space-for-time substitution for a water level change effect in CUNS2. Because carbonate concentrations of both cores, during the increase in carbonate, attain similar values, any water level-induced increase in CUNS2 carbonate seems at a minimum equalled by the effects of eutrophication.

In conclusion, the rapid increase in sediment accumulation rate and carbonate concentration is regarded mostly as a response to increased nutrients. This is further supported by an increase in core P concentrations. Finally, synchrony between eutrophication and increased throughflow is not necessarily controversial, as land drainage has historically been common

practice for improving agricultural land and therefore the simultaneity could be expected. While there are no historical records of drainage around Cunswick Tarn, Skelsmergh Tarn, a similar lake in the area, was drained in the late 19th century (Walker, 1955).

Decreasing core carbonate concentrations above depths of 26.5 cm (CUNS1) and 22.5 cm (CUNS2) are in conflict with the eutrophication signal of increased P and pigment concentrations as well as increased pelagic productivity. The decrease may in part be due to inhibition of calcite crystal formation, which occurs at high concentrations of organic matter and/or P (Reynolds, 1978; House, 1990; Danen-Louwerse et al., 1995; Hoch et al., 2000; Kasprzak et al., 2003). Collapsing charophyte populations, and therefore less carbonate-rich macrophytic fossil material, as indicated by macrofossil and historical data, would also lower core carbonate concentrations. Increases in the deposition of organic matter coupled with increases in productivity may also dilute carbonate content as eutrophication progresses, although this is less likely for Cunswick Tarn as the sedimentation rate is particularly high when sediment carbonate concentrations are maximal.

Combining all the aforementioned data, the stratigraphic zones are roughly divided in terms of productivity. Zone 1 represents a period of relatively low productivity as indicated by low concentrations of pigments, carbonate, P and cladocerans. However, zone 1a is characterised by slightly higher concentrations than zone 1b and likely covers a more productive phase in the lake. All indices suggest an increase in productivity through zone 2, with P showing the most consistent pattern. While concentrations of carbonate, pigments and cladocerans do not indicate increased productivity from zone 2 to zone 3 (though see Hann et al. (1994) and McGowan et al. (2005) regarding eutrophication-driven suppression of pigment and chydorid concentrations, respectively), increasing P concentrations and shifts in the biological communities show ecological effects of progressive nutrient enrichment. Therefore zone 3 is taken to represent the most eutrophic stage of the lake.

To better visualise the eutrophication- and climate-driven changes in the macrophyte and plankton communities, the reconstructions of the likely distribution of macrophytes and zooplankton through these stages (see Methods) are shown in Figure 4.23. These diagrams portray the gradual loss of macrophyte habitat through increasing light limitation combined with the recorded reduction in species diversity, leading to higher production in the plankton and increasing chemical stratification and concentrated biomass of macrophytes in the littoral

zone.

Cunswick Tarn

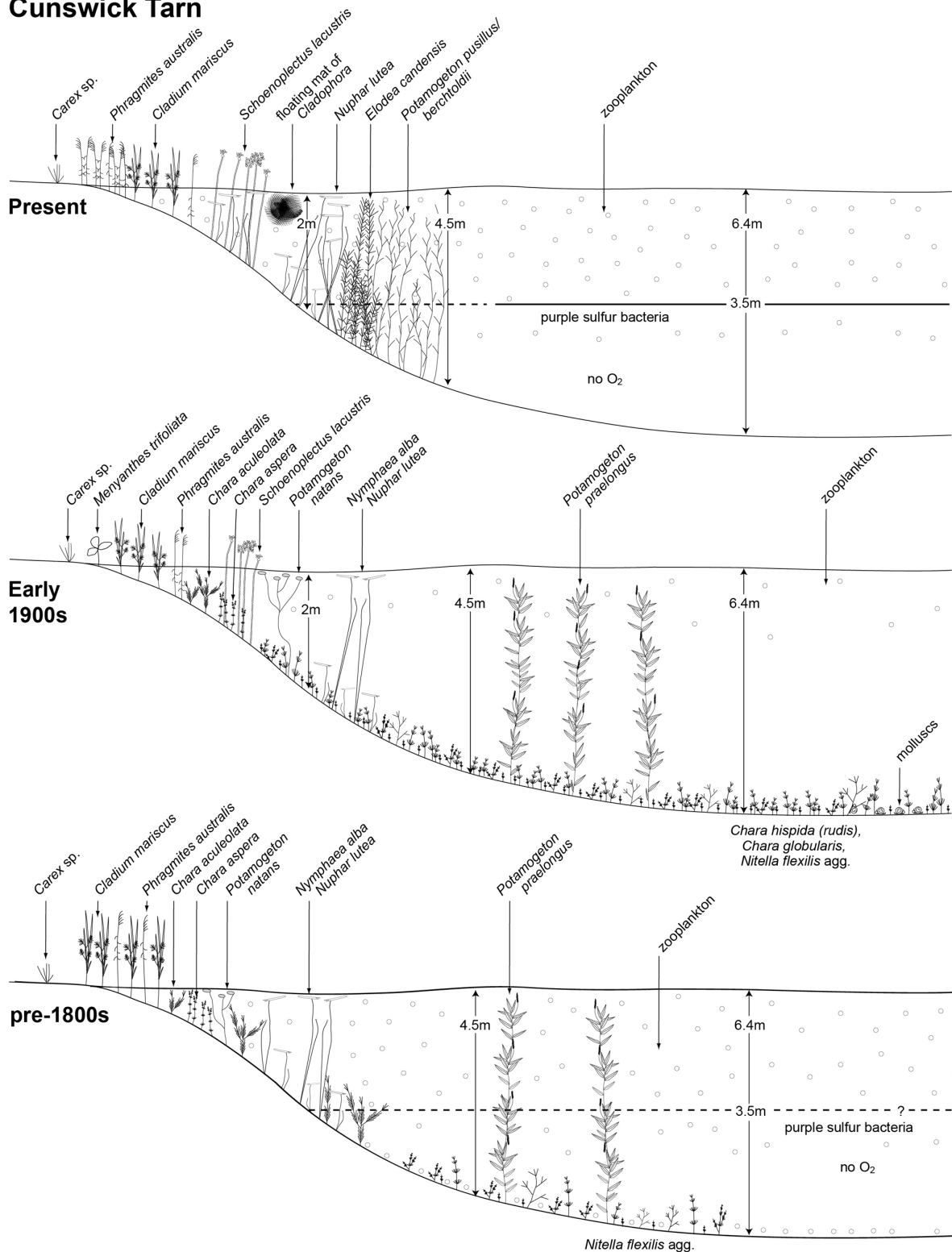


Figure 4.23: Cross sections of macrophyte cover, showing the currently dominant community, and historical communities based on available historical data.

4.5.2 Cunswick Tarn as a relatively unproductive lake

Overall, Cunswick Tarn was a relatively unproductive lake until the end of the 19th century. There are, however, important differences between zones 1a and 1b. Higher concentrations of cyanobacterial pigments and pelagic cladocerans (*Daphnia*, *Bosmina* spp.), and marginally higher carbonate deposition in zone 1a compared with zone 1b indicates elevated productivity. It is difficult to explain these differences, partly due to the absence of reliable dating for this section. Assuming the base of the core corresponds to the Mediaeval Warm Period, as indicated by extrapolation of sedimentation rates, higher temperatures rather than increased nutrient concentrations alone may have enhanced productivity compared with the Little Ice Age thereafter (see e.g. Wetzel, 1970; Schindler, 1978; Nolan et al., 1999; Kosten et al., 2009; Guilizzoni et al., 2012), including cyanobacteria (Gyllström et al., 2005). Although lakes in northern latitudes do not always show correlation between temperature and productivity (Anderson et al., 1996), similar changes have been observed in another site in northern England (Dong et al., 2012).

It is also possible that the core base reflects a period of early settlement and agricultural land management, leading to increased nutrient inputs. However, a relapse to lower productivity upcore would be unexpected if agricultural activity was a major factor, as in this area of England there is little evidence of farm abandonment or reduced agricultural activity due to the Little Ice Age (Johnson, 2003). Further, constrained analyses suggest cladoceran community change in this section of the core is not entirely driven by Ca and P, suggesting some other factors are more important. Therefore, the most likely explanation for increased productivity, specifically in the pelagic zone (Elliott, 2012), is warmer climate.

Both *Bosmina longirostris*/spp. and *Daphnia* spp. were abundant during this period, despite *Bosmina* usually being competitively excluded in lakes unless fish predation reduces the population of larger cladocerans (Ende and Dempsey, 1981). Notably, the abundance patterns of *Bosmina* and cf. *okenone* are similar (Figures 4.9, 4.12), suggesting a potential relationship between *Bosmina* presence and purple sulfur bacteria. Purple sulfur bacteria are associated with both the benthos and the metalimnion in freshwaters where anoxic, sulfidic habitats are exposed to sufficient light (Overmann, 2001). The presence of the same pigment in the uppermost core levels, and within the pelagic zone in the hypolimnion of Cunswick Tarn at present, suggests a pelagic habitat between anoxic and oxic layers may have existed in the

past. Relatively strong stratification is supported by the idea of a warmer climate in zone 1a, as higher temperatures create steeper temperature gradients in lakes, leading to higher thermal stability (Viner, 1985; Foley et al., 2012). The existence of hypolimnetic bacterial populations on one hand, and epilimnetic phytoplankton on the other, may have created niches to sustain coexistence of daphnids and bosminids, although both genera can feed on bacteria (Salonen and Lehtovaara, 1992). *Bosmina* has been shown to switch feeding habits in response to changes in food abundance (DeMott and Kerfoot, 1982), to subsist on poorer detrital food sources than *Daphnia* (Hessen et al., 1990), and to occupy hypolimnetic niches (Nykänen et al., 2009), indicating a potential for niche separation.

It could be argued that the absence of *C. obs/crys* below 87 cm truly reflects fish presence rather than failure to confirm presence of *C. obs/crys*. Following this hypothesis, the decline in *Bosmina* would indicate an early fish kill. However, this is unlikely given the increase in *Daphnia* as well as the large-bodied *Chydorus piger* towards the base of the core (e.g. White-side and Swindoll, 1988). Further, coexistence of *Bosmina* and *Daphnia* in fishless lakes has been observed for example in the Arctic (O'Brien et al., 1979) and in pond environments (Lynch, 1979; Dodson, 1979), and in Cunswick Tarn currently, showing coexistence is not conditional on fish presence.

The high abundance of *Bosmina longirostris*/spp. could also be an artefact of high water levels, with their decrease upcore reflecting decreasing water levels and a reduction in pelagic habitats. *Bosmina longirostris*, however, unlike other Bosminidae, is a species also associated with littoral zones and is therefore not an unambiguous indicator of water depth (Hofmann, 1998). Further, a few factors argue against significant water level changes. Firstly, the core base corresponds to a potential lake water level (5-5.5 m) lower than what would classify as unambiguously “deep” in terms of cladoceran habitats (Hofmann, 1998). Secondly, depths exceeding 6 m in Cunswick Tarn could serve as a “littoral” habitat, given the high potential colonisation depth of charophytes, and evidence of the typically deep-growing *Nitella flexilis* agg. in the lake in this period. Thirdly, littoral habitats are suggested by the abundance of plant-associated chydorids in the zone. Bosminidae in this core section are most likely to reflect food abundance in the water column.

In conclusion, the time period covered by zone 1a was relatively warm, and despite negligible or minor anthropogenic pressure, pelagic productivity was favoured in Cunswick Tarn, with

stratification and poor mixing promoting cyanobacterial communities (Figure 4.23). Marl-precipitating *Chara* cf. *aspera* were abundant in the shallower water, with *Nymphaea alba* and *Potamogeton natans* extending into deeper water. The deepest zones were characterised by more organic sediments and deeper-growing charophytes such as *Nitella* cf. *flexilis*. The variation of structural habitat, including areas of less dense macrophytes, maintained a relatively diverse community of pelagic, mud- and plant-associated cladocerans.

As climate deteriorated, Cunswick Tarn seems to have become better mixed and more oxygenated, as indicated by the decline of the sulfur bacterium (Figure 4.9). Productivity was depressed and the predominance of plant- or mud-associated chydorids indicates benthic- rather than pelagic-based food webs and therefore high water transparency. Macrophyte communities were similar to before (Characeae, *P. natans*), and a single *Potamogeton praelongus* seed found in CUNS1 adds to the image of a diverse *Potamogeton* zone at intermediate depths. Towards the end of the 19th century, Sphaeriidae and also other molluscs increased in abundance, especially in shallower water where carbonate deposition, as well as charophyte and nymphaeid abundance, increased. This could reflect climatic amelioration, but due to an absence of other changes indicated by core data, the pattern is difficult to explain.

4.5.3 Increased productivity at the end of the 19th century

Between 1904 and 1910, increasing productivity involved large changes in biological communities and carbonate precipitation (Figure 4.21), showing marl lakes can respond rapidly to nutrient increases. This initial change was followed by consistently high but variable carbonate deposition coupled with further shifts in biota. Peaks in carbonate deposition in 1910 and 1919, therefore, were not driven by the same biological communities, as also indicated by PCuA. Eutrophication induced stepwise changes in lake biota.

Initially, there was a dramatic increase in the abundance of charophytes as shown by fossil stem remains and oospores (Figures 4.13, 4.15). Identification of fossil oospore morphotypes in both sediment cores indicates that several *Chara* species existed in the lake. This is supported by historical records of *Chara curta*, *Chara aculeolata* and *Chara globularis* dating from 1899 and 1938. Carbonate precipitation, as is traditional in shallower marl lakes, was during these times driven by charophytes. Further, several factors suggest that plant beds were dense at this time. Firstly, the simultaneous increase in oospores and change in preservation pattern

of pigments suggesting sediment-level anoxia, implies dense macrophytic biomass (Lindholm et al., 2008; van den Berg et al., 1997). Secondly, cladocerans specifically associated with high macrophyte density were abundant (Davidson et al., 2007). Thirdly, there is a high abundance of epiphytic diatoms such as *Eunotia soleirolii* and *Gomphonema angustum* agg. (in synchrony with increases in diatom pigment concentrations) and macrophyte-associated molluscs. Finally, a lack of epipelagic diatoms suggests limited areas of open sediment, spatially and seasonally consistent with overwintering, dense *Chara* beds covering a large area of the benthos. Substantial carbonate precipitation and high competition for carbon and nutrients (also through epiphytes, see e.g. Strand and Weisner (2001) probably limited phytoplankton and pelagic cladocerans at this stage, as the abundance of pelagic cladocerans was very low (Figure 4.23).

It is peculiar that the carbonate peaks of 1910 and 1919 are mirrored by peaks in the chlorophyte - cyanobacterial pigment ratio in CUN2. As pigment data do not reveal species- or habitat-specific detail, the pattern is difficult to explain. As charophytes are chlorophytes, the 1910 peak coincident with high oospore numbers may be a result of high charophyte biomass and competitive suppression of cyanobacteria. However, this is unlikely to explain the 1919 peak as oospore and stem remains in CUN2 were decreasing at this time. Between these peaks, the abundance of plant-associated chydorids attained a maximum, likely reflecting a peak in plant density as numbers are commonly associated with macrophyte percent volume infestation (PVI) (Thoms et al., 1999; Gyllström et al., 2005; Davidson et al., 2007) and an abundance of suitable food (Sakuma et al., 2004), confirmed by a coincident peak in epiphytic diatom abundance. This would suggest that the 1919 chlorophyte:cyanobacteria + carbonate peak may have been driven by higher plants and microalgae rather than charophytes.

As carbonate deposition declined during the 1920s, charophytes continued to decline in abundance in favour of *P. natans*, *P. berchtoldii/pusillus* and nymphaeids. Concomitantly, cyanobacteria, chlorophytes and siliceous algae began to increase, and the epiphytic community underwent shifts towards diatom species associated with eutrophic waters and the cyanobacterium *Gloeotrichia* cf. *pisum*. *Gloeotrichia pisum* is a nitrogen-fixing colonial cyanobacterium that tends to be associated with linear-leaved submerged plants (John et al., 2002), and therefore peak abundance is in coherence with the abundance of leaf remains of *Potamogeton pusillus/berchtoldii* and canthaxanthin concentrations. These qualitative

changes occurred over a decade, which is relatively rapid. The rapidity may be explained by a shift from a positive benthic feedback involving charophyte productivity as outlined in the hypotheses, to a positive feedback of increasing phytoplankton/light limitation furthering the extinction of charophytes by angiosperms (Hargeby et al., 1994; Davidson et al., 2005; Ayres et al., 2008), leading to increasingly less benthic biomass. This shift also confirms the well established eutrophication-driven transition from predominantly submerged macrophytes to taller-growing and/or floating-leaved macrophytes, especially fine-leaved *Potamogeton* spp., (Davidson et al., 2002; Egertson et al., 2004; Davidson et al., 2005; Sand-Jensen et al., 2008; Davidson et al., 2008; Bennion et al., 2009, 2010; Salgado et al., 2010).

Although pelagic cladocerans increased slightly in abundance, Cunswick Tarn was still, in the 1920s and 1930s, dominated by benthic pathways and an abundance of macrophytes including charophytes sustaining clear water. Therefore, change occurred within previous habitats, rather than indicating a shift in lake state. However, while still chemically functioning like a marl lake and supporting charophyte communities, Cunswick Tarn was already exhibiting a fundamental ecological transition towards a more eutrophic system.

4.5.4 Transition to a more organic, non-marl lake system in late 1920s/early 1930s

Cunswick Tarn became ecologically and chemically less similar to marl lakes, and more similar to generic, nutrient-rich lakes towards the end of the 1920s, as shown by the decline in charophytes, carbonate precipitation, and associated biota. However, unlike the highly synchronous change in biological communities during the early increase in productivity at the beginning of the century, this later transition took place in steps over a few decades following the 1930s as reflected in the lower coherence of the principal curves of all communities.

The decline in plant-associated chydorids such as *Acroperus harpae* and *Pleuroxus laevis* was clearly not associated with a shift to a turbid state, as they became replaced by other plant-associated species such as *Sida crystallina*, *Alonella nana* and *Graptoleberis testudinaria* (Figure 4.11). This was synchronous to changes in the dominant macrophytes in Cunswick Tarn, suggesting cladocerans may show preference to particular macrophyte community structure. Indeed, similar species associations have been identified in other (shallow) lakes (Davidson et al., 2007), confirming the idea that cladocerans show responses to a multitude of biological

and chemical factors, making simple inferences less than robust (Hann et al., 1994; Lotter et al., 1998; Brodersen et al., 1998; Davidson et al., 2011).

Historical macrophyte records are more frequent from the time period from the 1930s to the present, and support the ecological change indicated by core data. For example, in the 1950s, the marl lake-associated emergent *Cladium mariscus* began to recede to the advantage of *Phragmites australis*, showing changes were taking place also within the lake margins. Where mechanical damage does not significantly interfere with reed growth (Schmieder and Pier, 2000), for example due to macrophyte loss and destabilisation of the water column and sediment (Madgwick et al., 2011), expansion of *P. australis* has been attributed to eutrophication (Mäemets and Freiberg, 2004; Liira et al., 2010), also at the expense of *Cladium mariscus* (Alvarez-Cobelas and Cirujano, 2007). Slightly later, 1969 marked the last historical record of *C. aculeolata*. Within this time frame, the palaeolimnological record shows decreases in mollusc abundance and sediment Fe content (suggesting redox-mediated Fe release), which may indicate decreasing oxygen concentrations.

The first historical records of *Elodea canadensis*, and the last of charophytes, are from the late 1970s/early 1980s. As *E. canadensis* reproduces exclusively vegetatively in the UK and senescing plants decay leaving no macrofossil record, presence/absence of *E. canadensis* before this time cannot be confirmed. However, because it is an invasive species in the UK, attention to its presence would have been expected during previous surveys such as a school trip survey by J. Birks in 1969. It is therefore likely that *E. canadensis* was a relatively recent introduction to the Tarn. Other species recorded in the 1980s include *C. hispida*, *Myriophyllum spicatum*, *Potamogeton natans*, *Potamogeton obtusifolius*, and *Potamogeton pusillus*. The fossil record complements these records, showing high abundance of Nymphaeaceae, and fine-leaved Potamogetonaceae. The Nymphaeaceae increase is likely to reflect an increase in *Nuphar lutea*, as *Nymphaea alba* remains become less frequent upcore and *Nu. lutea* is dominant at present. The *Ny. alba* - *Nu. lutea* transition with eutrophication has been noted in other lakes (Körner, 2001; Davidson et al., 2005; Ayres et al., 2008), reflected in historical UK habitats (Heslop-Harrison, 1955a,b).

As these charophyte-angiosperm shifts were taking place among the macrophyte community, mollusc abundance continued to decrease. Further, *Daphnia* abundance increased coupled with a slight increase in the mud-dwelling *Leydigia leydigi* and a decline in plant-associated

chydorids such as *Graptoleberis testudinaria* and *Sida crystallina*, coherent with patterns of increasing pelagic productivity reducing the quality and quantity of macrophyte habitats (e.g. Leavitt et al., 1994; Korponai et al., 2011), which may also have incurred negative changes in food web complexity (Rawcliffe et al., 2010). Cyanobacteria continued to increase in abundance, and planktonic diatom species indicative of eutrophic lakes became more frequent in the 1990s. During this succession, light penetration became sufficiently reduced to exclude charophytes from the lake, and *E. canadensis* became the dominant submerged macrophyte.

In the 21st century, purple sulfur bacteria either reappeared or became more frequent, as recorded by pigment analysis. Pigments of purple sulfur bacteria were present in hypolimnetic samples from the summer of 2011, confirming a planktonic habitat. This habitat is created by stable stratification due to strong chemical gradients in the lake associated with prevalent anoxic hypolimnetic conditions. It is therefore likely that the reappearance of cf. okenone marks increasing anoxia in Cunswick Tarn, consistent with very low sediment Fe concentrations. At present, Cunswick Tarn is dominated by *Daphnia* spp. and phytoplankton in the pelagic zone, and *N. lutea*, *E. canadensis* and *P. pusillus/berchtoldii* in the littoral zone, while molluscs are rare (Figure 4.23). This is in good agreement with the macrofossil record and the core top signal of increased pelagic productivity. Thus, with the exception of *E. canadensis*, the core top signal accurately portrays the current ecological state of the lake.

The similarity between CUN2 zone 1a and the core top is interesting. Even though both lake phases are characterised by pelagic productivity involving cyanobacteria and strong oxygen gradients promoting purple sulfur bacteria, the lake in its current state is much more eutrophic than in the past. This is reflected in the difference in macrophyte communities between the two phases. These specific differences and similarities were apparent also in the stratigraphic clusters and ordination diagrams, as unlike the other proxies, the core base macrofossil samples did not associate with core top samples. The lake at present does not support any species of charophyte, and *Ny. alba* has greatly decreased in abundance in contrast to the dominant *Nuphar lutea*. *P. praelongus* and *P. natans* have also disappeared. The current submerged/floating-leaved macrophyte species total of four is low compared with, for example, at least 9 spp. in 1983 (historical records) and 8 spp. in the early 1900s (conservative estimate based on collated core + historical data). The absence of fish has not prevented Cunswick Tarn from becoming a more pelagically driven lake even though ecological status at

given nutrient concentrations is often related to fish abundance and predation patterns (e.g. Hutorowicz and Dziedzic, 2008).

4.5.5 Impact on the understanding of marl lake ecology and eutrophication

The pre-enrichment biological communities in Cunswick Tarn were markedly different from those present during the eutrophication phase. Before increases in productivity, heavier marl precipitation coupled with dense charophyte (*Chara* cf. *aspera*) stands and abundant molluscs was restricted to the shallower (approximately <3 m) margins of the lake. Deeper water habitats were occupied by *Nitella* cf. *flexilis* and other charophyte species (*C. contraria*, *C. hispida* agg.), with relatively low chydorid abundance. *P. natans* and *P. praelongus* were growing amongst the charophyte meadows, creating a structurally diverse habitat. Productivity in the pelagic zone was very low, and the lake was in a clear-water state. This compares well with marl lakes currently of higher ecological status than Cunswick Tarn (Spence et al., 1984; Pybus et al., 2003).

Clear and rapid eutrophication-related changes in the charophyte community were predicted by the initial hypotheses. Also, it was predicted that biological communities would be driven both by core P and Ca concentrations (tested on subfossil cladoceran data), showing both changes relating to eutrophication, and changes relating to the carbonate chemistry of marl lakes. Both predictions are supported by the core data.

P and Ca are partly correlated due to the response of carbonate precipitation to increased productivity, and no attempt was made to explicitly separate the effects of the two variables. However, in ordination space, the large gradient in carbonate content showed community variation independent of P concentrations (Figure 4.22). This was largely driven by increasing abundances of *A. harpae* and *P. laevis* concomitant with decreasing contributions from other cladoceran taxa. The increase in carbonate precipitation was also associated with increases in molluscs, charophytes and epiphytic diatoms (Figure 4.18). All these taxa suggest a structurally diverse habitat (McAbendroth et al., 2005), with a food web fuelled mainly by macrophytes (see e.g. van den Berg et al., 1997) and therefore a strong benthic productivity component. Therefore, initial eutrophication responses were largely as predicted, although the rapidity of change was unexpected. Initial increases in carbonate precipitation (CUNS1: around 20 - >40%; CUNS2: <10 - >30%) and oospore numbers (CUNS1: >500; CUNS2:

>8000 100 cm⁻³) encompassed approximately 5 years.

The macrophyte community changed with eutrophication during the time period of high and fluctuating carbonate precipitation, shown in particular as a decrease in charophyte abundance and an increase in angiosperms. The charophyte - angiosperm shift also follows established eutrophication patterns, as predicted. However, it is important to note that charophytes were historically recorded in the lake after core data indicated reduced charophyte fertility and biomass, implying charophytes did not disappear from the site rapidly, but the decline rather took place over several decades, arguing against a rapid crash of charophytes in lakes with depth zonation.

Pelagic productivity remained low throughout the time of elevated carbonate precipitation. This supports statements regarding the sustenance of pristine submerged marl lake biota by the self-cleansing removal of nutrients and phytoplankton through carbonate precipitation (e.g. Wetzel, 1970; Siong and Asaeda, 2006; Robertson et al., 2007; Phillips et al., 2008). However, it is clear that marl lakes can reach a point where this self-sustenance no longer operates. The time period encompassing roughly 1919 and the late 1920s in Cunswick Tarn was marked by a sub-decadal rapid decline in core carbonate content, associated with changes in biological communities (Figure 4.21), involving increases in fine-leaved *Potamogeton* spp., replacement of diatom epiphytes by cyanobacterial epiphytes, and increases in pelagic productivity. These data suggest that, as carbonate precipitation declines, marl lakes can undergo rapid ecological shifts towards more pelagic productivity as recorded in other lake types (e.g. Scheffer et al., 1993), albeit in this case not characterised by an abrupt clear-turbid transition. Further, the increase in epiphytes throughout the 1910s and 1920s may have stressed macrophytes to the advantage of phytoplankton (Phillips et al., 1978).

Importantly, biological community shifts in Cunswick Tarn demonstrate that substantial ecological change can occur at relatively low nutrient concentrations. The current nutrient concentrations in Cunswick Tarn reflect eutrophy, but are modest compared with values recorded in more enriched lakes, yet the lake is superficially in near-complete resemblance to non-marl high-alkalinity lakes. Charophytes disappeared from the lake at some point between the early 1980s and the 21st century, corresponding to increases in Nymphaeaceae, pelagic cladocerans and cyanobacterial pigments. This indicates that charophytes were excluded through competition with angiosperms under increasing light limitation, again as initially predicted.

Both rapid and gradual responses were indicated by the biological communities and core chemical composition. Therefore, centennial-scale community-wide change was punctuated by periods of more rapid change. This indicates potential tipping points in marl lake ecology nested within more consistent responses, and whether or not regime shifts occurred during this progression is a matter of subjective interpretation of the concept of regime shifts. Although Cunswick Tarn has not undergone a clear-turbid transition, the colonisation depth of macrophytes has been reduced to 4-5 m. Rapid and monumental stepwise changes during the early 1900s led to a more gradual monotonic slip into an increasingly pelagic-dominated state of ecological degradation through the latter half of the century.

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5 Using $\delta^{13}\text{C}$ of cladocerans in lake eutrophication studies: results from contemporary and palaeolimnological research in high-carbonate lakes

5.1 Abstract

$\delta^{13}\text{C}$ analysis of cladoceran remains has been shown to be a promising method for palaeolimnological eutrophication studies, since cladocerans through consumption of phytoplankton reflect productivity and carbon isotope patterns in the plankton, and leave well-preserved chitinous remains in the sediment. However, cladoceran ephippia have not been investigated in stable isotope studies despite being required in lesser quantities than chitinous remains, and offering taxon-specificity. The applicability of ephippia $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{eph}}$) for tracing eutrophication was investigated using the sediment of a small calcareous lake, Cunswick Tarn (UK). The pattern of $\delta^{13}\text{C}_{\text{eph}}$ was compared against the eutrophication pattern revealed by multiple palaeolimnological proxies including plant and animal macrofossils, core carbonate content, pigments, diatoms and subfossil cladocerans. Supporting stable isotope data of modern lake waters and catchment soils, as well as the seasonal (April–September 2009 and 2010) $\delta^{13}\text{C}$ of cladocerans in Cunswick Tarn and two other calcareous lakes in the UK (Hawes Water, Malham Tarn) were also used. Rather than showing monotonic increases or decreases with eutrophication, $\delta^{13}\text{C}_{\text{eph}}$ appeared to follow patterns in the balance between macrophyte (benthic) and planktonic production, reflecting increasing ^{13}C use in the plankton under high macrophyte coverage and low pelagic zooplankton abundance. Further studies are required to establish whether these results are applicable on a wider scale.

5.2 Introduction

$\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of various components in lacustrine sediments have been used in palaeoenvironmental studies as proxies for temperature, hydrology and productivity (Leng et al., 2006). Increases in productivity affect the carbon cycle (and thus $\delta^{13}\text{C}$) in lakes in various ways; in high-carbonate lakes this includes increased biological uptake of both carbon dioxide (CO_2) and bicarbonate (HCO_3^-) from the dissolved inorganic carbon (DIC) pool, and the precipitation of calcite (CaCO_3) and therefore the interaction of inorganic and organic carbon cycling.

At equilibrium with the atmosphere, dissolved CO_2 and HCO_3^- have $\delta^{13}\text{C}$ values of approx-

imately -9 and $1-2$ ‰, respectively, compared with -8 ‰ of atmospheric CO_2 . Precipitation of CaCO_3 can be rapid and various disequilibrium fractionation values have been reported, while dissolution commonly occurs with minor, if any, fractionation (Darling et al., 2006). $\delta^{13}\text{C}$ fractionation values between gaseous CO_2 and precipitated CaCO_3 are temperature-dependent (Emrich et al., 1970; Romanek et al., 1992), with fractionation between aqueous HCO_3^- and precipitated CaCO_3 of approximately 1‰ (Romanek et al., 1992).

In natural environments, variability in the $\delta^{13}\text{C}$ of dissolved carbon is always observed owing to the interplay of differing sources of CO_2 and HCO_3^- such as catchment-derived respired and weathered dissolved carbon species, and the recycling of organic carbon. Allochthonous organic material, primarily derived in the UK from plants using the C3 photosynthetic pathway (certain crops such as maize are C4 plants) with fractionation values of -20 ‰, commonly has $\delta^{13}\text{C}$ values around -28 ‰ (Meyers and Ishiwatari, 1993). The influence of allochthonous input on $\delta^{13}\text{C}$ of in-lake material is likely to decrease with increasing lake size and trophicity (Grey et al., 2000; Post, 2002) but should be taken into consideration as a significant subsidy to food webs in smaller lakes (Pace et al., 2004).

During photosynthesis, ^{12}C is preferentially incorporated, and fractionation against ^{13}C by aquatic primary producers is variable compared with land plants. Fractionation values of -10 to -20 ‰ are common for phytoplankton when DIC is not limiting (Craig, 1953; Degens et al., 1968; Peterson and Fry, 1987; Descolas-Gros and Fontugne, 1990; Zohary et al., 1994; Gu and Schelske, 1996). Most rooted macrophytes can equally be assumed to reflect $\delta^{13}\text{C}_{\text{DIC}}$ processes in the water column as only isoetids seem to assimilate carbon from the sediment to a significant extent (Bowes and Salvucci, 1989). Generally, $\delta^{13}\text{C}$ of macrophytes is variable and lower than that of DIC (LaZerte and Szalados, 1982); under conditions not limited by boundary layer kinetics, values around -40 ‰ reflect uptake of CO_2 , and -13 ‰ of HCO_3^- uptake (Osmond et al., 1981). Thus, fractionation (and $\delta^{13}\text{C}$) values for phytoplankton and macrophytes can be very similar. While there are species-specific differences in phytoplankton $\delta^{13}\text{C}$, owing in part to differences in size and growth rate (Zohary et al., 1994; Yoshioka et al., 1994; Popp et al., 1998; Coletta et al., 2001), there are several biological and abiological processes that systematically direct the change in these values in lakes, in part dependent on lake morphometry, nutrient status and exposure.

DIC may become compartmentalised in low wind conditions and when a lake stratifies. In

these cases periods of high primary productivity and thus consumption of CO_2 for photosynthesis reduces the available carbon pool, resulting in use of the isotopically heavier HCO_3^- and/or a reduction in fractionation against $^{13}\text{CO}_2$ (e.g. Herczeg and Fairbanks, 1987; Hollander and McKenzie, 1991; Yoshioka et al., 1994; France et al., 1997; Vuorio et al., 2006). Both cases lead to higher $\delta^{13}\text{C}$ of both the DIC pool and photoautotrophs. On the other hand, particularly high productivity in this scenario can also be associated with lower $\delta^{13}\text{C}$. Large increases in pH result in a high proportion of HCO_3^- in the DIC pool, which creates a steep CO_2 gradient between the water and the atmosphere, resulting in intense drawdown of CO_2 (CED: Chemically Enhanced Diffusion). During CED, fractionation of CO_2 is enhanced and thus anomalously high proportions of ^{12}C diffuses into the lake (CEF: Chemically Enhanced Fractionation; CEF). This is most pronounced at pH approaching and exceeding 9, when fractionation exceeding -10‰ has been observed (Herczeg and Fairbanks, 1987; Bade and Cole, 2006). Thus, increased within-season productivity may also result in increasingly negative $\delta^{13}\text{C}$ of primary producers with important implications for the interpretation of $\delta^{13}\text{C}$, in particular in marl lakes where high pH and therefore high HCO_3^- concentrations are sustained (Pentecost et al., 2006).

In well-mixed lakes, DIC can be continuously replenished, resulting in negligible or very small seasonal changes in the $\delta^{13}\text{C}$ of phytoplankton (Gu and Schelske, 1996). In cases where allochthonous organic carbon input is substantial compared to autochthonous carbon demand, lower $\delta^{13}\text{C}$ values are sustained in the food web and $\delta^{13}\text{C}$ of particulate carbon can become lower with time during summer as phytoplankton become a more substantial component of particulate organic matter (POM) (Filstrup et al., 2009), therefore showing a negative, rather than a positive trend, over the productive season. Such patterns can therefore be expected in lakes under high allochthonous loading and in such cases bulk particulate carbon isotope analyses may give mixed information and more specific material should be analysed.

Lake sediments contain organisms deposited across all seasons, and can therefore give an integrated picture of catchment and in-lake $\delta^{13}\text{C}$ dynamics, typically weighted to the period of highest productivity where varves do not separate winter and summer seasons. Increasing $\delta^{13}\text{C}$ of organic and inorganic components through sediment cores reflecting increasing carbon limitation forms the basis for most interpretations of bulk sedimentary $\delta^{13}\text{C}$ profiles (Hodell and Schelske, 1998; Brenner et al., 1999; Hollander and Smith, 2001). However, changes in

bulk $\delta^{13}\text{C}$ along the length of a sediment core may conflict with predicted within-season trends of increasing $\delta^{13}\text{C}$ with increasing productivity. Multiple microbial processes can lead to low $\delta^{13}\text{C}$ in the water column and sediments, commonly involving close proximity of anoxic and oxic environments. Methanogenic bacteria produce methane with very low $\delta^{13}\text{C}$, and depending on the pathway, either leave a ^{13}C -enriched CO_2 pool or produce ^{13}C -depleted CO_2 , the latter which prevails in lacustrine environments (Whiticar, 1999). Methanotrophs subsequently produce substantial amounts of $^{12}\text{CO}_2$ (Hollander and Smith, 2001; Jones and Grey, 2011). However, in the apparent absence of methanotrophs, and/or a prevalence of the carbonate pathway, CO_2 with very high $\delta^{13}\text{C}$ resulting from the original methanogenic pathway may become significant, as methanogenesis has been used to explain high $\delta^{13}\text{C}_{\text{DIC}}$ in Lake Apopka (Gu and Schelske, 1996), and high $\delta^{13}\text{C}$ of dissolved carbonates in interstitial water of Lake Kinneret (Stiller and Magaritz, 1974). Sulfate-reducing bacteria in part compete for substrate with methanogenic bacteria (Whiticar, 1999). Where sulfate is reduced to sulfide, purple sulfur bacteria may become abundant, and typically have quite low $\delta^{13}\text{C}$ (Fry, 1986).

Provided microbially mediated ^{12}C is retained in the lake, organic matter may become progressively depleted in ^{13}C over the years. Substantial incorporation of hypolimnetic carbon by primary producers may also occur within a season in stratified lakes when the hypolimnion extends to the photic zone (Viner, 1985; Gervais, 2011; Hollander and Smith, 2001; Longhi and Beisner, 2009). In consequence, Hollander and Smith (2001) have put forward three scenarios of eutrophication-driven $\delta^{13}\text{C}$ change in sedimented organic and inorganic material that depend on the interplay of stratification, microbial processing, and photosynthetic activity, whereby eutrophication initially leads to decreasing $\delta^{13}\text{C}$, with positive changes in $\delta^{13}\text{C}$ above a eutrophication threshold.

It becomes clear, through the aforementioned examples, that interpretation of bulk $\delta^{13}\text{C}$ is problematic without comprehensive, supporting data, which are often impossible to produce retrospectively. Palaeolimnological analyses of compound-specific $\delta^{13}\text{C}$ may overcome many of the problems associated with bulk analyses and can answer more specific palaeolimnological questions (Hollander and Smith, 2001; Neunlist et al., 2002; Enders et al., 2008)

Herbivorous cladocerans as a phytoplankton $\delta^{13}\text{C}$ proxy

Trophic fractionation of ^{13}C is thought to be very small, and thus organisms with feeding

phases corresponding to food source productivity phases can reflect baseline isotope conditions (Post, 2002). The $\delta^{13}\text{C}$ of herbivorous zooplankton and phytoplankton have been found to be most similar in meso-eutrophic lakes (Grey et al., 2000), however, due to various feeding adaptations, data consisting of amalgamated zooplankton samples may be misleading and introduce scatter (Grey et al., 2000; Matthews and Mazumder, 2003). Specific studies on cladocerans feeding on phytoplankton (and periphyton) may be expected not to reflect the $\delta^{13}\text{C}$ of POM and instead provide accurate information of seasonal and year-to-year photoautotrophic carbon dynamics in lakes (Visconti and Manca, 2011; Boll et al., 2012). However, *Daphnia* $\delta^{13}\text{C}$ have also been shown to reflect ingestion of bacteria, which might complicate food web assumptions where bacteria are abundant (Fry, 1986; Jürgens et al., 1994; Burns and Schallenberg, 2001; Feuchtmayr et al., 2004).

Cladocerans in temperate climates have a variable, subannual lifespan, and numbers are usually greatest in summer when offspring are produced in response to plentiful food (Hebert, 1978; George and Hewitt, 1999; George, 2011). Due to rapid reproductive responses to food resources and growth through adulthood, cladocerans collected at sub-season intervals during summer can also reflect more short-term changes in phytoplankton $\delta^{13}\text{C}$. Further, ephippia are produced in pulses usually once or twice in a season as a response to, for example, the fall in temperature at the end of summer, crowding, food limitation or fish predation (Kleiven et al., 1992; Pijanowska and Stolpe, 1996; Alekseev and Lampert, 2001; Ślusarczyk, 2001). As the effects of carbon uptake evolve throughout the summer season, the $\delta^{13}\text{C}$ signal from ephippia may be particularly useful in tracking eutrophication in palaeolimnological studies. Cladoceran exoskeletal remains have been shown to be subject to only minor taphonomic changes in $\delta^{13}\text{C}$ (Perga, 2011), and show promise as a palaeolimnological tool (Perga, 2010). Ephippia are easier to pick and are not required in as high a quantity as exoskeletal remains, thereby allowing both relatively effortless and importantly, taxon-specific analysis (Davidson & Leng, unpubl.).

The aim of this study was twofold. Firstly, to track seasonal variation in *Daphnia* spp. $\delta^{13}\text{C}$ in three high-carbonate lakes of contrasting nutrient status and morphology. Second, supported by these data and the results of a parallel palaeolimnological study (Chapter 4), to test the applicability of $\delta^{13}\text{C}$ of *Daphnia* spp. ephippia as a tracer of eutrophication-induced change in the primary productivity of the most eutrophic of these lakes, Cunswick Tarn, over the last

few hundred years. It was hypothesised that there would be observable and consistent change in $\delta^{13}\text{C}$ during summer, and specifically positive changes in the mesotrophic lakes. Also, a null hypothesis of increasing $\delta^{13}\text{C}$ with increasing lake trophy was adopted. Therefore, it was expected that eutrophication-related changes in other palaeolimnological data of Cunswick Tarn (Chapter 4) would be reflected by simultaneous increases in fossil ehippia $\delta^{13}\text{C}$. values relating to eutrophication? Because of the possibility of CED and CEF, and microbial loops, in Cunswick Tarn, it was difficult to predict the direction of change in $\delta^{13}\text{C}$ over summer.

5.3 Methods

5.3.1 Study sites

Cunswick Tarn

Cunswick Tarn is a small, fishless, eutrophic kettlehole lake in Cumbria, UK (Table 5.1, Figure 5.1a). It has maximum and mean depths of 6.4 and 3.7 m. The relatively small catchment is comprised mainly of improved pasture and to a lesser extent woodland. The lake receives carbonate-precipitating spring water low in nutrients from the wooded slope to the east, and the main inflow is a stream at the southeast end (Figure 5.1b). Water with high phosphorus concentrations seeps into the lake from the woods and pastures to the north. In addition, a drain pipe from the north pasture feeds directly into the lake and the soil at the lake margin is eroding. A low-gradient, occasionally still, outflow is located at the southwest end (Figure 5.1b). The profile of the outflow would seem to suggest that it may be artificial and lake levels may therefore have been changed in the past. The site is very sheltered and waves form only in very strong winds. The lake is currently in an ecologically unfavourable state. Steep oxygen gradients developing in the summer (Figure 5.2), combined with the limited areas of shallow water, result in a substantial volume of hypoxic water. Macrophytes are abundant but of low diversity. Phytoplankton are also abundant and develop blooms in summer. Historical plant survey data as well as a palaeolimnological study of the site (Chapter 4) reveal that lake biology and chemistry have changed dramatically over the last two centuries.

Hawes Water

Hawes Water is a small mesotrophic kettlehole lake in Silverdale, Lancashire (Table 5.1). It

Table 5.1: Summary data for the study sites; RT = retention time. Single values of pH, TP and chl *a* are the means of two years (2009, 2010) of data covering all seasons, and TN was measured once, in January 2011.

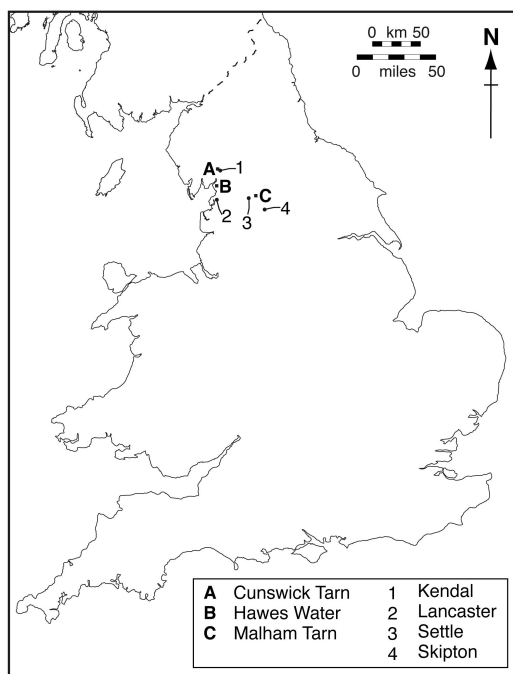
Site name	UK grid ref.	County	Area (ha)	Altitude (m asl)	Z_{\max} (m)	Z_{mean} (m)	RT (yr)	pH	pH (range)
Cunswick Tarn	SD 489937	Cumbria	0.8	138	6.4	3.7	NA	8.2	7.5 - 9.1
Hawes Water	SD 477766	Lancashire	5.7	8	12.2	4.2	0.32	8.2	7.9 - 8.6
Malham Tarn	SD 893667	N.Yorkshire	60	375	4.4	2.4	0.33	8.4	7.7 - 8.8

	TP ($\mu\text{g L}^{-1}$)	TP (range)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Chl <i>a</i> (range)	TN (mg L^{-1})
Cunswick Tarn	55.8	22.3 - 154	84.0	1.0 - 328	1.32
Hawes Water	19.7	5.7 - 40.3	11.8	4.3 - 27.9	0.77
Malham Tarn	17.5	9.1 - 34.4	6.4	1.1 - 15.8	0.58

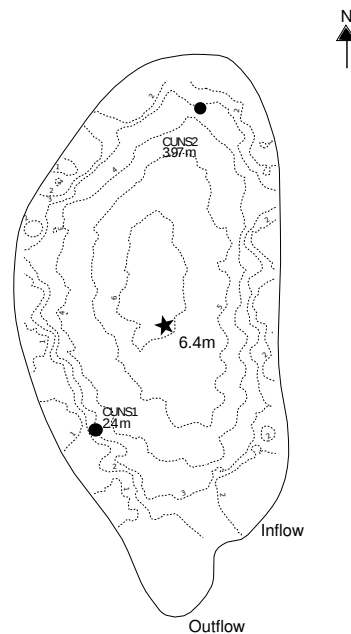
lies in a shallow basin of Carboniferous limestone in the Gait Barrows Nature Reserve. The catchment is 1.7 km² and consists, in addition to the nature reserve, of holiday lets, pastures and permanent homes. Hawes Water has mean and maximum depths of 4.2 m and 12.2 m, respectively. The lake receives water through an artificial inflow connected to Little Hawes Water to the north, and from spring seepage from the surroundings as well as the lake bed. There is an artificial outflow with associated land drains at the southern end of the lake leading to Hawes Water Moss, the creation of which in the 19th century resulted in lowered water levels. Consequently, given the steeply shelving bathymetric profile of the lake, there is only a limited surface area of shallow water (Figure 5.1c). The lake has a retention time of 0.32 years. Stratification takes place between May and September with anoxia developing below a depth of approximately 7 m (Figure 5.3). Macrophytes colonise the margins, to a depth of approximately 5 m only, and the pelagic zone is dominant. Marl precipitation is prevalent through summer, and marl coating overwintering charophytes persists through all seasons.

Malham Tarn

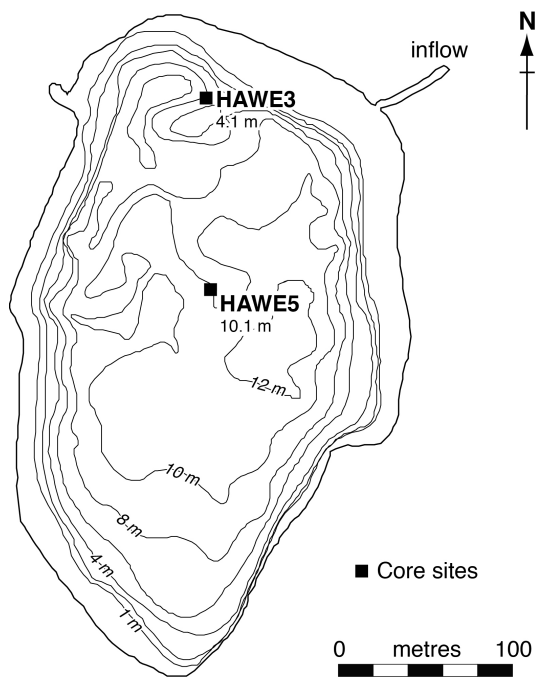
Malham Tarn lies in Craven, North Yorkshire. It is a shallow mesotrophic lake with a mean depth of 2.4 m, a maximum depth of 4.4 m and an area of 60 ha (Figure 5.1d). At 375 m asl, it is the highest marl lake in the UK and forms part of a National Nature Reserve noted for nationally scarce species and habitat diversity. The tarn is designated a Site of Special Scientific Interest, a RAMSAR site as well as a Special Area of Conservation. The 11.5 km²



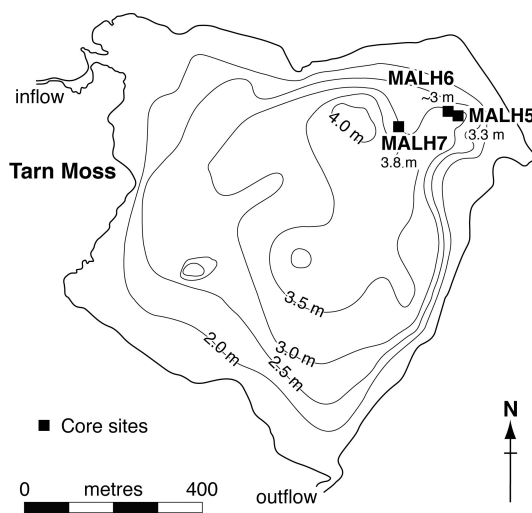
a The locations of the study sites on a map of Great Britain.



b Cunswick Tarn



c Hawes Water



d Malham Tarn

Figure 5.1: Location and bathymetric profiles of the three study sites.

catchment consists of a mixture of improved and natural cattle and sheep pastures, scattered farms, and the Field Studies Council centre lying on the north flank.

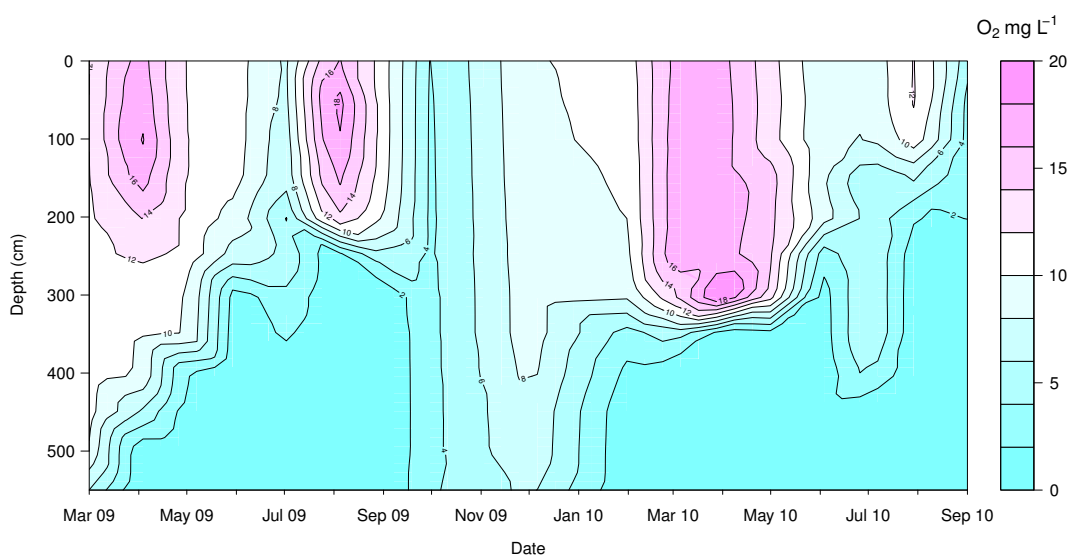


Figure 5.2: The depth profile of oxygen in Cunswick Tarn through years 2009 and 2010.

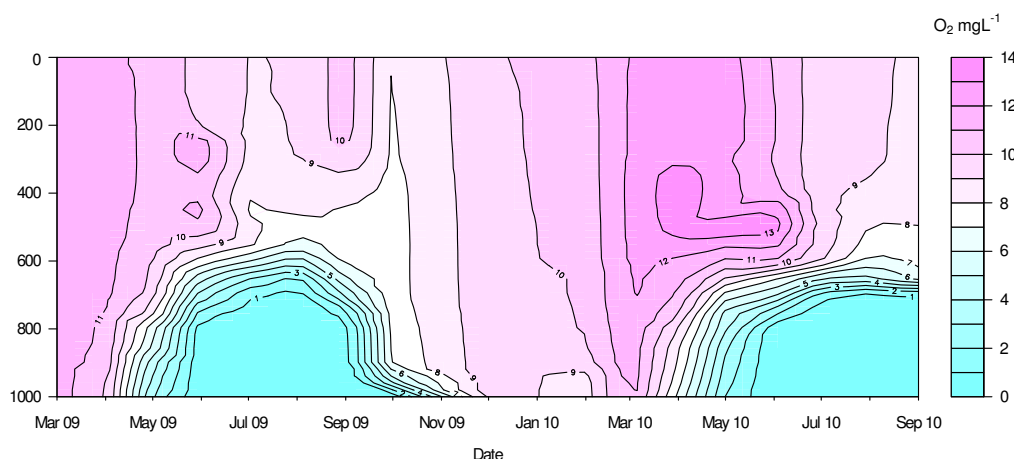


Figure 5.3: The depth profile of oxygen in Hawes Water through years 2009 and 2010.

The lake is exposed, with limited sheltering to the north by a wooded slope. Dominant and often strong southwesterly winds maintain a well mixed water column throughout the year. The main inflow is a large stream at the northwestern end, but the lake also receives water from springs to the north. The outflow to the south is controlled by a weir. The estimated retention time is 0.33 years, but flushing rate varies seasonally. Charophytes colonise the whole lake where substrate is favourable (thereby excluding certain shores), and the pelagic zone is limited. Carbonate precipitation is induced particularly at times when *Chara* spp. are abundant, recorded as a draw-down of in-lake DIC and a decrease in water calcium concentrations (Pentecost, 1984; Coletta, 2004).

5.3.2 Core collection, chronology and lithostratigraphy

A littoral sediment core (CUNS2; 94 cm) was collected from Cunswick Tarn (Figure 4.3) in October 2009 in the northern margins at a water depth of 4 m using a “Big Ben” piston corer (internal diameter 140 mm) (Patmore et al., *in prep*). Visible changes in sediment colour or structure in the core were noted in the field. The core was extruded at 1 cm intervals.

CUNS2 was dated radiometrically with ^{210}Pb , a naturally produced radionuclide derived from atmospheric fallout (termed unsupported ^{210}Pb) with a half-life of 22.3 years. Sediment ^{210}Pb concentration profiles can yield accurate stratigraphic dates for the past 150 years. ^{137}Cs (half-life 30 years) and ^{241}Am (half-life 432.2 years) are artificially produced radionuclides, deposited as atmospheric fallout from nuclear weapons testing and nuclear reactor accidents, and were used as markers to secure the chronology indicated by ^{210}Pb data.

Freeze-dried sediment samples were analysed for ^{210}Pb , ^{226}Ra , ^{137}Cs and ^{241}Am by direct gamma assay in the Bloomsbury Environmental Isotope Facility (BEIF) at University College London, using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra using the 295 keV and 352 keV gamma rays emitted by its daughter isotope ^{214}Pb following three weeks of storage in sealed containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 keV and 59.5 keV (Appleby et al., 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self-absorption of low energy gamma rays within the sample (Appleby et al., 1992).

Carbonate and organic matter content were quantified using the loss on ignition (LOI) procedure following Dean (1974). Weights after combustion were recorded as percentages of dry weight. For percentage carbonate, the weight lost was first multiplied by 1.36 to account for the difference in molecular weight of CO_2 and CO_3^{2-} .

5.3.3 Collection of contemporary samples

Cladocerans were sampled in all three lakes from April to September in 2009 and 2010. Due to adverse weather, May and August 2009 data from Malham Tarn are missing. Samples consist of five vertical trawls along an open-water transect, performed by lowering a phytoplankton

net (mesh size 100 μm) to the maximum attainable depth at each point. Littoral samples consist of three tube samples (approximately 80 cm length, internal diameter 71 cm) collected from three representative locations around each lake. Upon collection, samples were preserved in methylated ethanol until subsampling in 2011 and 2012. Gut clearance was not part of the procedure as the method is advisable mostly for copepods (Feuchtmayr and Grey, 2003).

Additional material was analysed for $\delta^{13}\text{C}$ from Cunswick Tarn. Macrophytes, *Cladophora* sp., a mixed phytoplankton + zooplankton sample, as well as soil from around the lake, were collected in June and July 2010 and analysed for $\delta^{13}\text{C}$. Sediment was posted to the NERC Isotope Geosciences Laboratory, British Geological Survey, Nottingham (UK), immediately upon collection. Plant material was freeze-dried before postage. In August 2011, a sample representing phytoplankton was collected. First, water was prefiltered through a 50 μm mesh net in order to remove larger particles including zooplankton. This water was then passed through a 1.2 μm pore size WhatmanTM ashed GF/C filter that was subsequently freeze-dried before analysis.

Surface (arm's depth) and hypolimnetic (below 4 m) water samples from the centre of the lake and surface samples of the main inflow were collected from May to September 2010 for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ analysis in order to understand the seasonal hydrology of Cunswick Tarn. Further, $\delta^{13}\text{C}$ of surface water was analysed through this time period and January 2011, and of inflow water in August.

In August 2011, water for $\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analysis was collected at 1 m intervals between the surface and 5 m and finally 5.5 m in order to investigate the nature of chemical stratification in the lake. Water from the main inflow was also collected for the same analyses.

5.3.4 Sample preparation

In Cunswick Tarn and Malham Tarn, *Daphnia* spp. were abundant enough to subsample for analysis. However, this was not the case in Hawes Water where mainly *Bosmina* spp. but also *Ceriodaphnia* spp. were subsampled instead. This limits comparison between sites, but for some months in Malham Tarn and Hawes Water, more than one genus were subsampled to check for any indication of species-specific differences. Ehippia of *Daphnia* spp. in months of abundance were separated and collected in order to investigate potential $\delta^{13}\text{C}$ differences. There were no cladocerans of sufficient abundance in the April 2009 Cunswick Tarn sample

probably due to a smaller sampling volume caused by equipment failure.

All cladoceran material was picked by tweezers or needles under a dissecting microscope and soaked in deionised water for a few hours before rinsing in a sieve. For samples undergoing HCl treatment to remove carbonates, soaking was followed by rinsing with 5% HCl before the deionised water rinse. The default method was to apply HCl treatment to all fossil samples, but not to contemporary samples as the latter are less likely to be coated with carbonates. However, due to the calcareous nature of all lakes and possible treatment effects, replicates with and without HCl treatment were compared (Perga, 2010). Within-treatment variability was also assessed by replicates due to the possibility of methodological issues (Brodie et al., 2011).

Sufficient sample size required 100 *Bosmina* spp., 1 ephippium, and depending on size, 15 to 40 *Daphnia*/*Ceriodaphnia* spp. Whenever possible, multiple ephippia were analysed in order to capture a reasonable average. This was achieved for all contemporary samples, but not for all CUNS2 levels. Further, some core levels contained only *Simocephalus* spp. in sufficient abundance, and therefore, both taxa were picked from selected core levels to investigate suitability of amalgamation.

After washing, samples were picked into tin vials and dried overnight in a drying cabinet at no higher than 45°C. Thereafter, they were posted in multiwell trays to the NERC Isotope Geosciences Laboratory for analysis.

5.3.5 Laboratory analyses

All stable isotope analyses were performed at the NERC Isotope Geosciences Laboratories. $\delta^{13}\text{C}_{\text{DIC}}$ analyses were performed by phosphoric acid treatment of BaCO_3 precipitated in the laboratory. After precipitation of the bicarbonate, the material was filtered, washed with deionised water and dried at 40°C and ground in agate. The carbonate was reacted with anhydrous phosphoric acid in vacuo overnight at a constant 25°C. The CO_2 liberated was separated from water vapour under vacuum and collected for analysis. Measurements were made on a VG Optima mass spectrometer. Overall analytical reproducibility for these samples is normally better than 0.1‰ for $\delta^{13}\text{C}$ (2σ). Isotope values ($\delta^{13}\text{C}$) are reported as per mil (‰) deviations of the isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) calculated to the VPDB scale using a within-run laboratory standard calibrated against National Bureau of Standards (NBS) standards.

Water $\delta^{18}\text{O}$ was analysed by equilibration with CO_2 using an Isoprep 18 device for oxygen isotope analysis with mass spectrometry performed on a VG SIRA. For water $\delta^2\text{H}$ analysis, an on-line Cr reduction method was used with a EuroPyrOH-3110 system coupled to a Micromass Isoprime mass spectrometer. Isotopic values ($\delta^{18}\text{O}/\delta^2\text{H}$) are reported as per mil (‰) deviations in relation to the International standard, VSMOW2 (Vienna Standard Mean Ocean Water 2). Analytical precision is typically $\pm 0.08\text{‰}$ for $\delta^{18}\text{O}$ and $\pm 1.0\text{‰}$ for $\delta^2\text{H}$.

For small organic samples (catchment soils and core sediments [every 5 cm of CUN2], cladocerans, ephippia, plant materials), %C and $\delta^{13}\text{C}$ were measured using a Carlo Erba 1500 elemental analyser. %C and %N analyses (as weight; from which C/N was calculated) involved calibration against an Acetanilide standard. $\delta^{13}\text{C}$ analyses were performed on-line to a VG TripleTrap (with a secondary cryogenic trap in the mass spectrometer for very low carbon content samples) and Optima dual-inlet mass spectrometer, with $\delta^{13}\text{C}$ values calculated to the Vienna Pee Dee Belemnite (VPDB) scale using a within-run laboratory standard (BROC1) calibrated against NBS-19 and NBS-22. Replicate analysis of well-mixed samples indicated a precision to within 0.1% (‰C) and 0.1‰ (1 SD) ($\delta^{13}\text{C}$).

Stable isotope analyses of CUN2 sediment CaCO_3 were performed on samples (5 cm intervals) gently disaggregated in 5% sodium hypochlorite solution (10% chlorox) for 24 hours to oxidise reactive organic material. Samples were then washed three times in distilled water and sieved at 85 μm to remove any ostracod valves. The <85 μm fraction was filtered, washed with deionised water and dried at 40°C and ground in agate. The isolated material was reacted with anhydrous phosphoric acid in vacuo overnight at a constant 25°C. The CO_2 liberated was separated from water vapour under vacuum and collected for analysis. Measurements were made on a VG Optima mass spectrometer. Overall analytical reproducibility for these samples is normally better than 0.1‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (2σ). Isotope values ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$) are reported as per mil (‰) deviations of the isotopic ratios ($^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$) calculated to the VPDB scale using a within-run laboratory standard calibrated against NBS standards.

5.3.6 Data analysis

Cladoceran stable isotope sample preparation treatments (HCl vs no HCl, variation of number of ephippia) were compared by t-tests, paired where appropriate. The seasonal isotope data series did not cover a sufficient number of data points for time series analysis. Therefore,

correlations between $\delta^{13}\text{C}$ of cladocerans and in-lake chlorophyll *a* concentration, cladoceran (+ copepod) abundance, alkalinity and pH were tested by linear regression including month and year as variables to investigate potential temporal effects. Variance Inflation Factors were computed to test for collinearity of terms. Following regression with multiple terms, ANOVA was run comparing models with and without the resulting significant variables to isolate the effect and p value of such variables. All analyses were performed using R version 2.12 (R Development Core Team, 2010).

The positive relationship between dissolved oxygen and photosynthesis also leads to a positive relationship between oxygen concentrations and primary productivity (del Giorgio and Peters, 1994; Staehr and Sand-Jensen, 2007; Balmer and Downing, 2011). Therefore, oxygen concentrations were also used as an indicator of productivity in the study sites. Balmer and Downing (2011) demonstrated that net efflux of CO_2 is likely above oxygen saturation of 95%, and therefore this value is displayed as reference in the seasonal oxygen data series.

Core CUNS2 $\delta^{13}\text{C}$ data were corrected for the atmospheric depletion of ^{13}C resulting from the combustion of fossil fuels and deforestation (the Suess effect) (Vermeer et al., 2007) using the equation of (Schelske and Hodell, 1995). Corrected values for years preceding 1840 are likely to have high error, and further, the radiometric dating profile for CUNS2 terminates at 1867, therefore no correction was applied to $\delta^{13}\text{C}$ values for core levels below 46.5 cm.

5.4 Results

5.4.1 Cunswick Tarn water isotope data

Chemical stratification in Cunswick Tarn during summer months is reflected in the depth distribution of $\delta^{13}\text{C}_{\text{DIC}}$ in August 2011, with values becoming lower at depths below the oxycline at approximately 3 m (Figure 5.4). Epilimnetic values are approximately -12‰ , while the lowest hypolimnetic value is $<-17\text{‰}$. The time series of surface $\delta^{13}\text{C}_{\text{DIC}}$ shows a generally decreasing pattern between May and August, with the highest value above -9‰ recorded in May and the lowest value of $<-14\text{‰}$ recorded in September 2010 during the breakdown of stratification. The single winter $\delta^{13}\text{C}_{\text{DIC}}$ value of January 2011 is similar to that of the previous September. Inflow $\delta^{13}\text{C}_{\text{DIC}}$ was only sampled twice, in August 2010 and 2011. The value was lower in 2011 (-13.5‰) than in 2010 (-11.9‰). Also, unlike in 2010, inflow $\delta^{13}\text{C}_{\text{DIC}}$ in 2011 (-13.5‰) was lower than that of lake surface water (-12.1‰) (Figure 5.5).

$\delta^{18}\text{O}$ and $\delta^2\text{H}$ data of the epilimnetic, hypolimnetic and inflow water are shown in Figure 5.6.

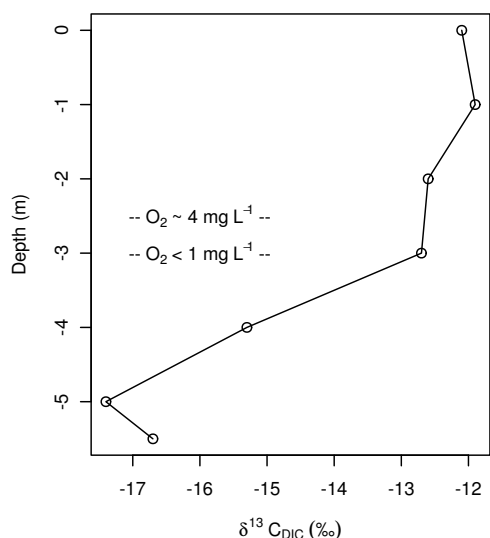


Figure 5.4: The depth profile of $\delta^{13}\text{C}_{\text{DIC}}$ in August 2011. Below approximately 3 m, the lake is hypoxic.

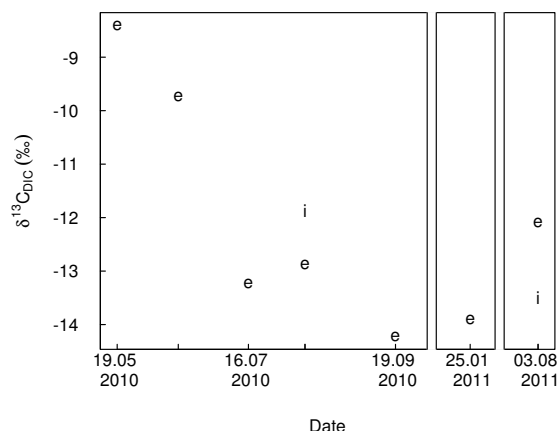


Figure 5.5: Epilimnetic (e) and inflow (i) $\delta^{13}\text{C}_{\text{DIC}}$ data.

Inflow and hypolimnetic values generally plot close to the global meteoric water line (GMWL) whereas epilimnetic values plot close to (and slightly below) the GMWL, with values becoming higher later in the summer. Hypolimnetic and inflow values are generally lower than epilimnetic values, with an exceptionally low inflow value in July (-7.4‰). August 2011 values from all depths, and the inflow, were higher than in August 2010.

5.4.2 Cunswick Tarn soil and plant analyses

C/N values of soil material are variable (Table 5.2) - pasture soils have slightly lower C/N (< 15) than woodlands (> 15). The reed bank has a slightly higher C/N value than the north pasture located upslope, while the bare soil bank immediately leading into the lake has a value more similar to that of the woodlands. For aquatic plants, C/N values are highest for emergent plants with more structural tissue (*P. australis* 20.3, *S. lacustris* 47.2), and lowest for the sample representing zooplankton, phytoplankton and other POM (11.3). The alga *Cladophora* sp., growing as floating, coarse mats, also has a high C/N value (31.8), while the submerged species have intermediate values. The range of values in aquatic plants is broader than that of soils (Figure 5.7).

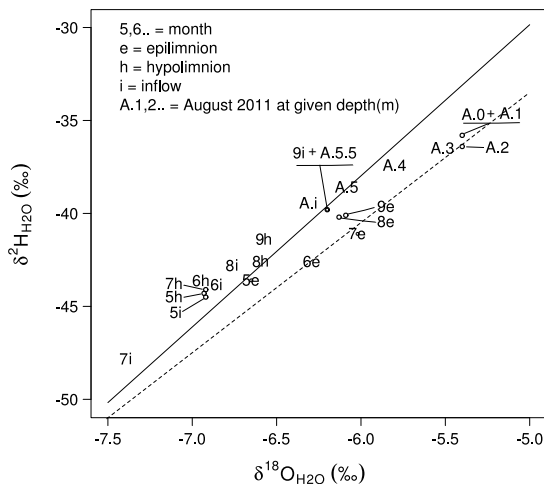


Figure 5.6: $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of lake water as ‰ VS-MOW2; all data are from 2010 apart from August 2011, labeled separately. Where values are closely clustered, labels have been moved and connected to points. Solid line: Global Meteoric Water Line, dashed line: UK Meteoric Water Line

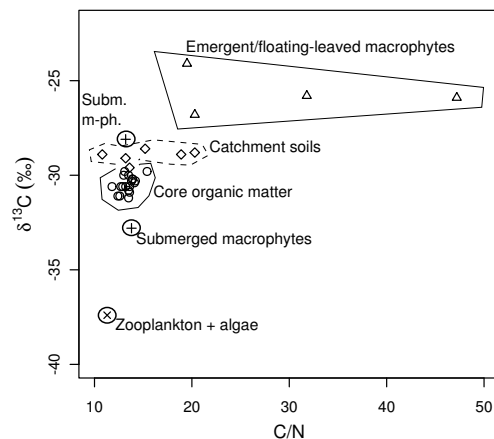


Figure 5.7: Biplot of $\delta^{13}\text{C}$ against C/N for selected materials in and around Cunswick Tarn.

Soils have $\delta^{13}\text{C}$ signatures typical of C3 plant-based soils. The emergent plants *Phragmites australis* and *Schoenoplectus lacustris*, the floating-leaved species *Nuphar lutea* and the floating mass of the alga *Cladophora* sp. also have $\delta^{13}\text{C}$ values within the range commonly found in land plants with the C3 pathway (Meyers and Ishiwatari, 1993). The submerged species *Elodea canadensis* and *Potamogeton pusillus* as well as the mixed sample of POM, zooplankton and phytoplankton have lower $\delta^{13}\text{C}$ values more similar to aquatic organisms using dissolved carbon (Meyers and Ishiwatari, 1993). The $\delta^{13}\text{C}$ of the mixed and phytoplankton samples of August 2011 are -35.9 and -36.5 ‰, respectively.

The relationship between $\delta^{13}\text{C}$ and C/N distinguishes sample types (Figure 5.7). Emergent- and floating-leaved macrophytes show a tendency to have high $\delta^{13}\text{C}$ and high, variable C/N values, while catchment soils have comparatively lower and unchanging $\delta^{13}\text{C}$ values over a narrower range of C/N. The two samples of submerged macrophytes are intermediate in $\delta^{13}\text{C}$, with low and similar C/N values, and zooplankton + algae have the lowest $\delta^{13}\text{C}$ and C/N values.

Table 5.2: Elemental and isotopic data of catchment soil sampled in May, and emergent, floating-leaved, and submerged macrophytes as well as POM sampled in June and July 2010 in Cunswick Tarn

Sample	$\delta^{13}\text{C}(\text{‰})$	%C	%N	C/N
Pasture (W)	-29.6	18.4	1.3	13.6
Pasture (N)	-28.9	5.6	0.5	10.8
Woods 1	-28.8	44.3	2.2	20.3
Woods 2	-28.6	21.5	1.4	15.2
Soil bank	-28.9	52.0	2.8	18.9
Reed bank	-29.1	37.6	2.9	13.2
<i>Phragmites australis</i>	-26.8	43.8	2.2	20.3
<i>Schoenoplectus lacustris</i>	-25.9	44.3	0.9	47.2
<i>Nuphar lutea</i>	-24.1	41.2	2.1	19.5
<i>Cladophora</i> sp.	-25.8	34.8	1.1	31.8
<i>Elodea canadensis</i>	-28.1	42.2	3.2	13.2
<i>Potamogeton pusillus/berchtoldii</i>	-32.8	45.3	3.3	13.8
Phyto- & zoo-plankton, POM	-37.4	49.6	4.4	11.3

5.4.3 Seasonal cladoceran isotope data series

There was no significant difference between the acid treatment and deionised only-treatment of contemporary cladoceran material. Within-sample variability tested for two sets of five replicates (May and August 2010, Cunswick Tarn) yielded standard deviations of 0.37 and 0.21‰, respectively. Between-species variability was inconsistent - for Malham Tarn in April 2009, *Bosmina* were 0.9‰ higher than *Daphnia*, but this reversed in June (difference 3.3‰). For Hawes Water in August 2010, *Bosmina* were 3‰ higher than *Daphnia*. In contrast, the $\delta^{13}\text{C}$ of *Ceriodaphnia* and *Bosmina* in July 2010 in Hawes Water were within 1‰ of each other.

Differences in the $\delta^{13}\text{C}$ of *Bosmina* and *Daphnia* may in part be a reflection of size-dependent feeding constraints (Geller and Müller, 1981; Hessen, 1985), and in part of feeding habits (see e.g. DeMott and Kerfoot, 1982; Hessen et al., 1990; Salonen and Lehtovaara, 1992). In Malham Tarn in April 2009, when the values were similar, *Bosmina* were relatively large and size difference was not a factor. However, $\delta^{13}\text{C}$ differences were not consistent between sites where size differences were apparent. Owing to these discrepancies, $\delta^{13}\text{C}$ of *Bosmina* in Hawes Water are not compared with *Daphnia* $\delta^{13}\text{C}$ in absolute terms.

Ephippia were subsampled from adult *Daphnia* on three occasions. May 2010 values of adults

and ehippia in Malham Tarn were identical. However, for June 2009 ehippia were 3.5‰ lower. For June 2009 in Cunswick Tarn, ehippia were 0.6‰ lower. More adults than ehippia were required for sufficient sample size, and therefore differences may in part be due to variability among adults rather than adult-ehippial fractionation only.

Within-lake cladoceran $\delta^{13}\text{C}$ values were similar between years (Figure 5.8). Malham Tarn tended to have the most positive values, followed by Hawes Water and Cunswick Tarn. The range of values was also largest in Malham Tarn, spanning 11.5‰ compared with 8.8 and 7.0‰ for Hawes Water and Cunswick Tarn, respectively. In Malham Tarn, April values were below -35‰ , rising through the summer. The maximal value of -26.9‰ was attained in August 2010, followed by a similar value in September. The September value in 2009, on the other hand, was much lower - unfortunately, there are no data from August 2009.

The lowest $\delta^{13}\text{C}$ values through the season in Hawes Water were, similarly to Malham Tarn, recorded in April and early May and are around -40‰ . Thereafter, they increased to approximately -35‰ , displaying only minor variability. Cunswick Tarn displays a slightly different pattern, with lowest values recorded in June and September in both years (although there are no data for April 2009). Most values fall between -37 and -40‰ . There is no clear relationship between $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{13}\text{C}_{\text{Daphnia}}$ in the lake.

Linear regression (adjusted $R^2 = 0.81$) followed by ANOVA for Cunswick Tarn gave significance to chlorophyll *a* (positive correlation; $p < 0.01$) and *Daphnia* abundance (negative correlation; $p < 0.05$) as explanatory variables of $\delta^{13}\text{C}_{\text{Daphnia}}$ (Figure 5.9). For Hawes Water, month (positive correlation; $p < 0.05$) and alkalinity (negative correlation; $p < 0.05$) were significant, with an adjusted $R^2 = 0.71$. For Malham Tarn, only month came out as a significant predictor, although the regression result may be a chance outcome (overall $p > 0.05$).

5.4.4 Core CUN2 bulk organic and inorganic analyses

Neither C/N nor organic matter $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{org}}$) show strong trends through the core profile of CUN2 (Figures 5.7, 5.10). Variation within uncorrected $\delta^{13}\text{C}_{\text{org}}$ is limited to 1.4‰ with values generally higher below a core depth of 30 cm and lower above this limit, mostly scattering between -30 and -31‰ . However, following correction for the Suess effect, an increasing trend in $\delta^{13}\text{C}_{\text{org}}$ is apparent upcore of 15 cm with the uppermost sample almost 2‰ higher than the uncorrected value. C/N values lie between 11.8 and 15.4, the maximum being one

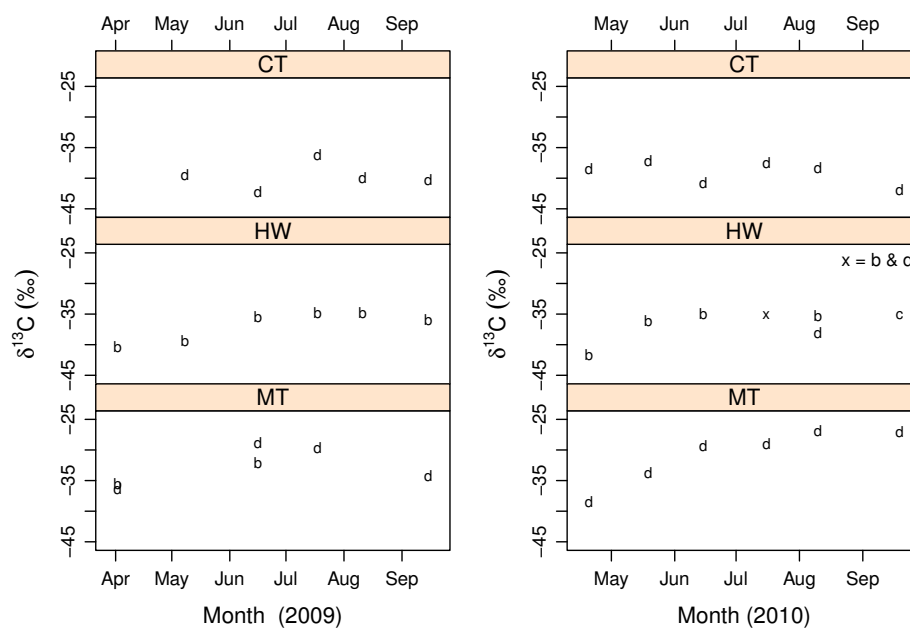


Figure 5.8: Seasonal $\delta^{13}\text{C}$ data from 2009 and 2010 of the three study sites Cunswick Tarn (CT), Hawes Water (HW) and Malham Tarn (MT) shown on the same scale. “d”, “c” and “b” refer to *Daphnia*, *Ceriodaphnia* and *Bosmina*, respectively.

distinct peak at 65.5 cm and the minimum a peak at 30 cm. Most values lie between 12.6 and 14.2, and a tendency for lower values upcore may be present.

Carbonate content was not sufficient to produce stable isotope data from a depth of 50 to 70 cm. Below 40 cm carbonate $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{carb}}$) values are out of equilibrium with rainfall and are also relatively variable, fluctuating between -3 and -6‰ . Above 40 cm, values are

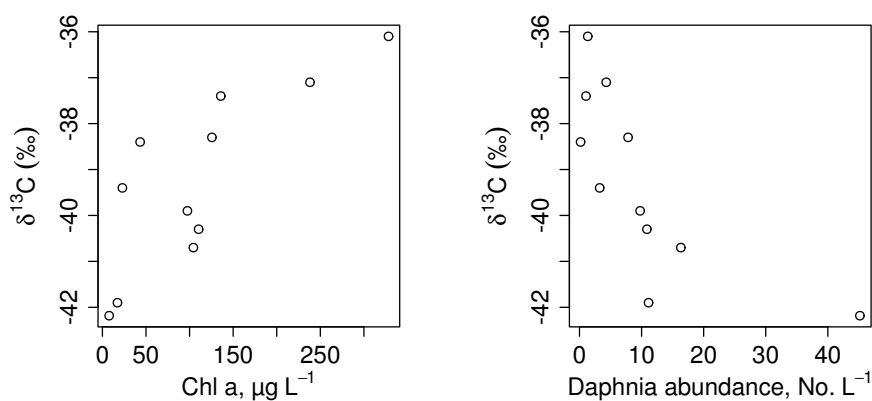


Figure 5.9: *Daphnia* $\delta^{13}\text{C}$ data of Cunswick Tarn displayed against *Daphnia* abundance and chl *a* concentration.

less variable and in equilibrium with rainfall, between -6 and -7‰ . There is a tendency for $\delta^{18}\text{O}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{carb}}$ to covary except between 40 and 45 cm. $\delta^{13}\text{C}_{\text{carb}}$ is variable below 25 cm, fluctuating between -7.3 and -11‰ . Above this depth, uncorrected values fluctuate only between -7.9 and -8.7‰ , with corrected values increasing upcore to a maximal value of -5.9‰ .

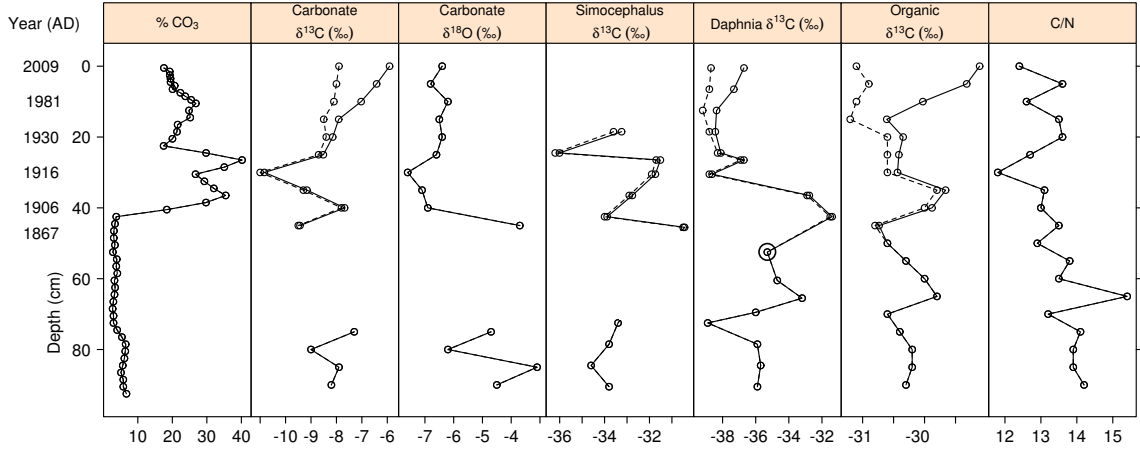


Figure 5.10: CUNS2 data of bulk material and *Simocephalus* + *Daphnia* ephippia; missing carbonate and *Simocephalus* isotope data are due to insufficient carbonate and ephippia in the sediment, respectively. The double-circled *Daphnia* data point at depth 52.5 cm is an amalgamation of depths 45.5 and 59.5 cm. Dashed lines indicate data prior to correction for the Suess effect (Schelske and Hodell, 1995).

5.4.5 Core CUNS2 analyses of fossil ephippia

Varying the number of ephippia analysed had no consistent effect on $\delta^{13}\text{C}$ values. There was no significant difference between the sets in terms of range or mean (Table 5.3). Similarly, acid treatment seemed to have no effect on the $\delta^{13}\text{C}$ values of the uppermost core level samples. The acid-treated set had lower variability, but given the large variability in the other acid-treated samples this is probably not a function of the acid treatment itself. Nevertheless, acid treatment was applied to all subsequent core samples because any effect of residual carbonates would be more likely in the sediment samples with higher carbonate content than those analysed in the trial. All $\delta^{13}\text{C}$ values from the core top fall within the range of $\delta^{13}\text{C}$ of *Daphnia* in 2009, the autumn of which core CUNS2 was recovered. Variability within core levels was considerable (e.g. sd of samples from 6.5 cm = 2.0‰).

Daphnia ephippia were abundant within the uppermost 27 cm (Figure 5.11). Downcore, re-

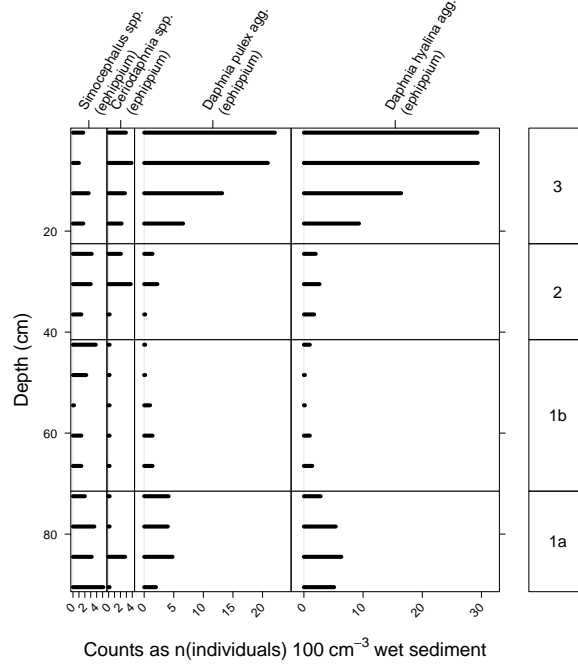


Figure 5.11: CUNS2 macrofossil analysis ephippia counts.

mains were sparse and only one and two ephippia were analysed for levels 42.5 and 60.5 cm, respectively. Ephippia were amalgamated from levels 45.5 and 59.5 cm. Abundance was higher again between 72.5 cm and the core base. There were not sufficient *Simocephalus* ephippia for analysis in the uppermost 17 cm, and between 42 and 68 cm. Only one ephippium was analysed from levels 26.5 and 60.5 cm, and two from levels 36.5 and 72.5 cm.

Table 5.3: Trial CUNS2 core $\delta^{13}\text{C}_{\text{eph}}$ data from depth 0.5 cm (first two) and 6.5 cm (rest). The number of replicates for each set is 5 and 3, respectively.

n(ephippia)	Mean $\delta^{13}\text{C}$	Min $\delta^{13}\text{C}$	Max $\delta^{13}\text{C}$	Range	Acid
5	-38.7	-41.2	-36.10	5.1	no
5	-38.7	-39.4	-37.80	1.6	yes
3	-39.5	-41.0	-36.1	5.7	yes
5	-37.9	-38.4	-37.2	1.2	yes
10	-39.5	-41.5	-37.6	3.9	yes

The range of $\delta^{13}\text{C}_{\text{eph}}$ for all core samples is 7.7‰ (*Daphnia*) and 5.7‰ (*Simocephalus*) and relatively high. For *Daphnia*, data suggest three separate stratigraphic sections (Figure 5.10). From the core base to 69.5 cm, $\delta^{13}\text{C}_{\text{eph}}$ are relatively low and around -36‰ except for one lower value of -38.9‰ at 72.5 cm. From 65.5 cm to 36.5 cm, $\delta^{13}\text{C}_{\text{eph}}$ are higher, ranging from -35.3 (amalgamated 52.5 cm sample) to -31.5‰ (42.5 cm). From 30.5 cm to the core top,

$\delta^{13}\text{C}_{\text{eph}}$ become again lower, and are less variable, ranging from -39.2 to -36.7‰ (including values both corrected and uncorrected for the Suess effect).

Due to lack of a consistent data sequence of *Simocephalus ehippia*, and high variability of $\delta^{13}\text{C}_{\text{eph}}$, trends cannot be evaluated. However, $\delta^{13}\text{C}_{\text{eph}}$ are consistently higher than corresponding $\delta^{13}\text{C}_{\text{eph}}$ of *Daphnia* in the lower and upper core sections (Figure 5.10). For the two matching core depths in the section where *Daphnia* $\delta^{13}\text{C}_{\text{eph}}$ are relatively high, there either is no difference (36.5 cm) or *Daphnia* $\delta^{13}\text{C}_{\text{eph}}$ are higher by 2.5‰ (42.5 cm).

5.5 Discussion

5.5.1 Hydrological processes in and around Cunswick Tarn

Inflow $\delta^{13}\text{C}_{\text{DIC}}$ reflects primarily soil respiration-derived HCO_3^- but also the dissolution of carbonates in the catchment (Leng and Marshall, 2004). Given the relatively large difference between inflow $\delta^{13}\text{C}_{\text{DIC}}$ values in August 2010 (-11.9‰) and 2011 (-13.5), it is likely that source $\delta^{13}\text{C}_{\text{DIC}}$ is changeable. Early summer 2010, when low water levels were noted, was relatively dry, with 34% less rainfall recorded between May and July 2010 than 2011 (Newton Rigg weather station). As spring water $\delta^{13}\text{C}_{\text{DIC}}$ usually reflects catchment processes with a time lag, the higher $\delta^{13}\text{C}_{\text{DIC}}$ value of 2010 may reflect earlier water stress and less soil respiration, resulting in the carbonate dissolution signal being more dominant (Coletta, 2004). Because, at both sampling occasions, lake surface $\delta^{13}\text{C}_{\text{DIC}}$ was not consistently lower or higher than that of the inflow, it is likely in-lake processes are dominant in driving seasonal $\delta^{13}\text{C}$ changes in the lake.

$\delta^{18}\text{O}$ and $\delta^2\text{H}$ values of lake water correspond more to the GMWL than the line suggested for the UK (Darling and Talbot, 2003) (Figure 5.6). Hypolimnetic water shows a tendency to become isolated from the lake surface as evidenced by the tight clustering of values especially in the earlier summer months (Figure 5.6). The very low value of the inflow in July 2010 is not reflected in that of the epilimnion. As July was a very rainy month, the inflow value may reflect the “amount effect” of more intense rain events resulting in lower $\delta^{18}\text{O}$ (Darling and Talbot, 2003). In general, however, all $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values lie within the range of within-month variability of rainfall (Darling and Talbot, 2003). Surface water data points reflect slight evaporation, especially during and following drier months. Overall, however, Cunswick Tarn is not and has not recently been an evaporatively driven lake, as the core top $\delta^{18}\text{O}_{\text{carb}}$

values are in equilibrium with temperature- related rainfall. Below a core depth of 40 cm, however, more closed conditions are indicated, firstly through highly variable non-equilibrium $\delta^{18}\text{O}_{\text{carb}}$ (Leng et al., 2006) and secondly, through the similarity in the patterns of $\delta^{18}\text{O}_{\text{carb}}$ and $\delta^{13}\text{C}_{\text{carb}}$ (Talbot, 1990) (Figure 5.10).

On the other hand, disequilibrium $\delta^{18}\text{O}$ values may also result from *Chara* carbonate deposits (Coletta et al., 2001; Pentecost et al., 2006) as well as from rapid carbonate precipitation in highly eutrophic conditions (Teranes et al., 1999). While the former can not be ruled out owing to palaeolimnological evidence for *Chara* carbonate precipitation in the littoral zone for all core levels below 40 cm, the latter is unlikely owing to relatively low concentrations of pigments and carbonate in these core levels indicating productivity lower than present (Chapter 4). A change from closed to open conditions in Cunswick Tarn around a core depth of 40 cm (late 1800s/early 1900s) is the most likely explanation for the $\delta^{18}\text{O}$ pattern, as agricultural land was commonly improved by digging drainage ditches. Supporting this idea, Skelsmergh Tarn, a similar lake nearby, was drained at some point in the 19th century (Walker, 1955).

5.5.2 Sources of organic carbon to Cunswick Tarn

The C/N values throughout core CUNS2 are intermediate to values expected from organic matter derived from land plants and algae (Meyers and Ishiwatari, 1993), and comparable to the values of the soils sampled around the lake as well as those of submerged macrophytes (Figures 5.7 ,5.10). Therefore, it is likely that the accumulated organic matter is a mixture of direct erosion of sediment from immediate surroundings, other allochthonous material, and finally autochthonous material. The decreasing C/N values towards the core top may be an indication of increased algal productivity but variability and the small range of change prevent any firm conclusions from being drawn. $\delta^{13}\text{C}_{\text{org}}$ values fluctuate within a relatively small range, and are similar to those of submerged macrophytes and surrounding soils (Figure 5.7). Again, this probably reflects deposition of a mixture of allochthonous and autochthonous material (Meyers and Ishiwatari, 1993), but given the large ecological change in Cunswick Tarn over time the absence of a clear trend in $\delta^{13}\text{C}_{\text{org}}$ is somewhat surprising. Substantial eutrophication-driven changes have been shown under similar or even smaller concentrations of total phosphorus (TP), both in a positive, (Hodell and Schelske, 1998; Brenner et al., 1999; Lu et al., 2010; Neumann et al., 2002; Torres et al., 2012), and a negative (Teranes and

Bernasconi, 2005; Hollander and Smith, 2001), direction (although ambivalent $\delta^{13}\text{C}$ changes did occur in a eutrophic lake forming part of the study by Teranes and Bernasconi (2005)). The consistent increase in Suess-corrected values since the 1950s may reflect more recent eutrophication in agreement with the null hypothesis. Because Cunswick Tarn is such a small lake, and continues to support abundant macrophytes, it is probable that both allochthonous and macrophytic material have in part masked changes in pelagic productivity despite increasing eutrophication, and bulk organic $\delta^{13}\text{C}$ is a poor measure of eutrophication in such circumstances.

5.5.3 Processes driving $\delta^{13}\text{C}$ of primary producers in Cunswick Tarn

At present, Cunswick Tarn has both abundant macrophytes and phytoplankton during summer months. Macrophytes experience a relatively early dieback around September/October and were not found overwintering with the exception of small *E. canadensis* turions. Phytoplankton, on the other hand, maintain productivity through a larger part of the year with surface chlorophyll *a* values between $5\ \mu\text{g L}^{-1}$ and $13\ \mu\text{g L}^{-1}$ during winter months, and exceeding $30\ \mu\text{g L}^{-1}$ in early spring peaks. Summer values between 100 and $300\ \mu\text{g L}^{-1}$ have been recorded. Therefore overall carbon demand is expected to be high. Nevertheless, expectations of increasing $\delta^{13}\text{C}$ over summer months, and generally high $\delta^{13}\text{C}$, as under the null hypothesis, were not met. $\delta^{13}\text{C}$ of macrophytes, POM, phytoplankton and *Daphnia* were low throughout the summer period (Table refplantsoils, Figure 5.8). Further, surface water $\delta^{13}\text{C}_{\text{DIC}}$ became lower over summer, which is opposite to what would be expected when ^{12}C is preferentially removed from the water column by biota (Gu and Schelske, 1996). The value in May is closest to that expected of CO_2 in equilibrium with atmospheric CO_2 , while those from July onwards are more akin to water containing substantial respired carbon (Gu et al., 1999) (Figure 5.5). Given the large allochthonous carbon input, slow drainage, relatively thin layer of oxygen-rich water of the lake, and finally, decreasing $\delta^{13}\text{C}_{\text{DIC}}$ values with increasing depth in the hypolimnion (Figure 5.4), substantial amounts of respired $^{12}\text{CO}_2$ are highly likely to explain this pattern.

Respired carbon may be directly and indirectly used by both phytoplankton and zooplankton in the lake. Firstly, *Daphnia* may be migrating to the hypolimnion in summer as implied by the abundance of red individuals with high concentrations of haemoglobin (pers. obs),

which has been coupled with hypolimnetic feeding (Salonen and Lehtovaara, 1992). Secondly, while the oxycline is variable between years and months, for the most part light sufficient for photosynthesis reaches the hypoxic zone, providing a potential habitat for phyto- and bacterioplankton. Thirdly, hypolimnetic water develops a sulfurous smell in summer, which in combination with the presence of purple sulfur bacteria confirmed through pigment analysis from August 2011 samples, suggests bacterial loops through sulfur bacteria may be sustained in the food web. Such bacteria typically have low $\delta^{13}\text{C}$ values and can form a part of zooplankton diets (Fry, 1986). Bacteria overall can form a considerable fraction of *Daphnia* diets (Jürgens et al., 1994; Burns and Schallenberg, 2001; Feuchtmayr et al., 2004; Taipale et al., 2008), which may contribute to the consistently low $\delta^{13}\text{C}_{\text{Daphnia}}$ in the lake. Unfortunately no night-time O_2 measurements were taken. However, it is highly likely respiration increases in the epilimnion during low irradiance, and respired carbon may therefore be in temporarily abundant supply in the epilimnion regardless of diffusion or vertical migration by plankton (Markager and Sand-Jensen, 1989).

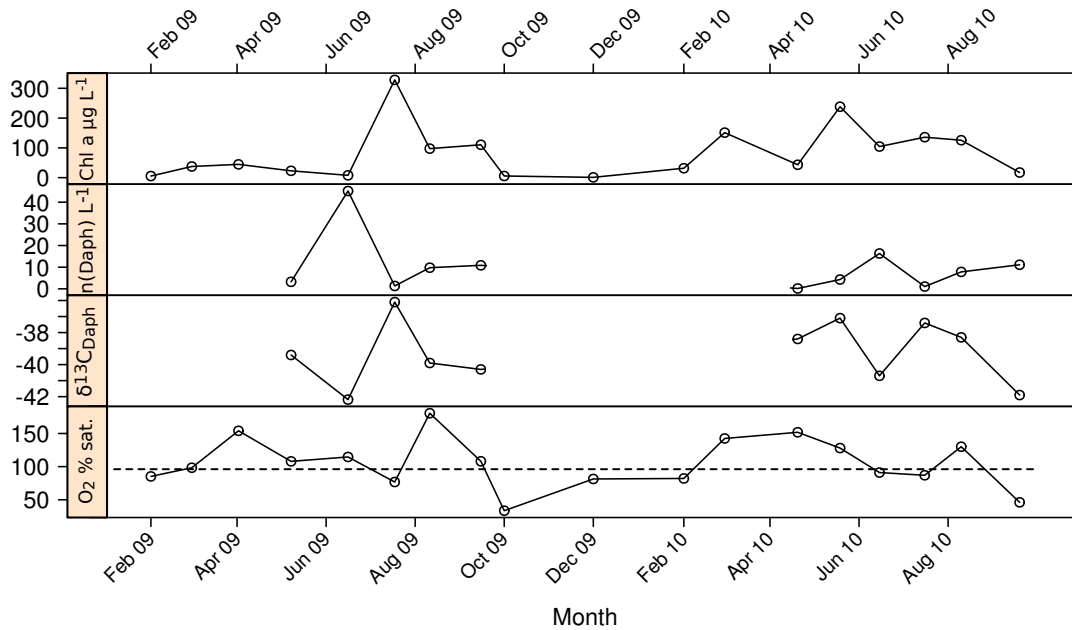


Figure 5.12: Cunsick Tarn: Productivity, indicated by abundance of *Daphnia* and chl *a*, and oxygen saturation, with $\delta^{13}\text{C}_{\text{Daphnia}}$.

Respired ^{12}C is not the only driver of $\delta^{13}\text{C}$ through summer, as indicated by change in $\delta^{13}\text{C}_{\text{Daphnia}}$ independent of $\delta^{13}\text{C}_{\text{DIC}}$. Although robust analysis was prevented by the insuf-

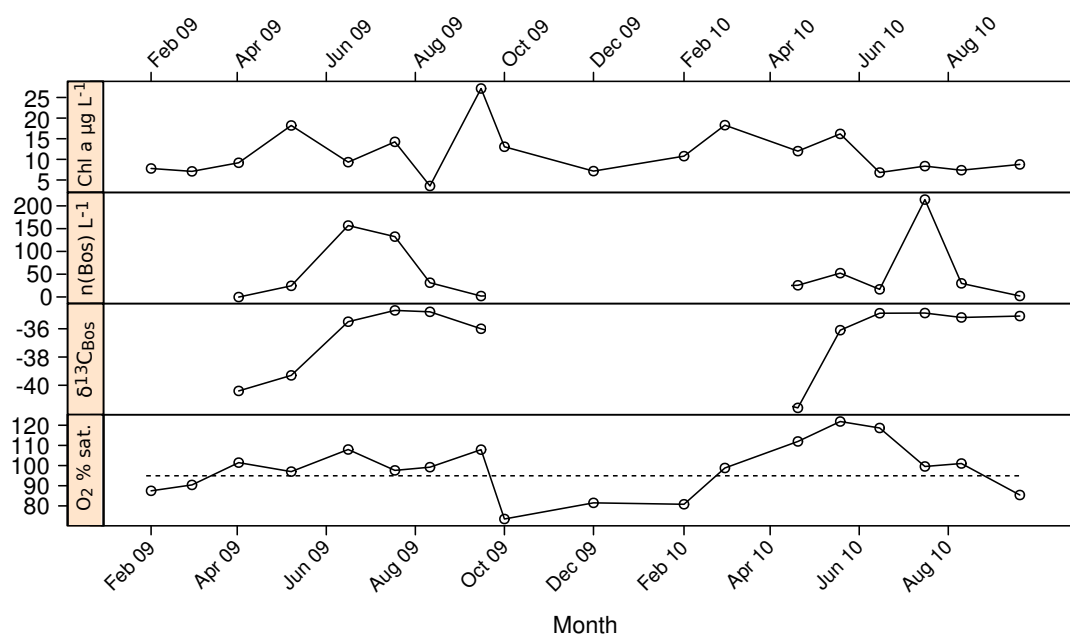


Figure 5.13: Hawes Water: Productivity, indicated by abundance of *Bosmina* and chl *a*, and oxygen saturation, with $\delta^{13}\text{C}_{\text{Bosmina}}$.

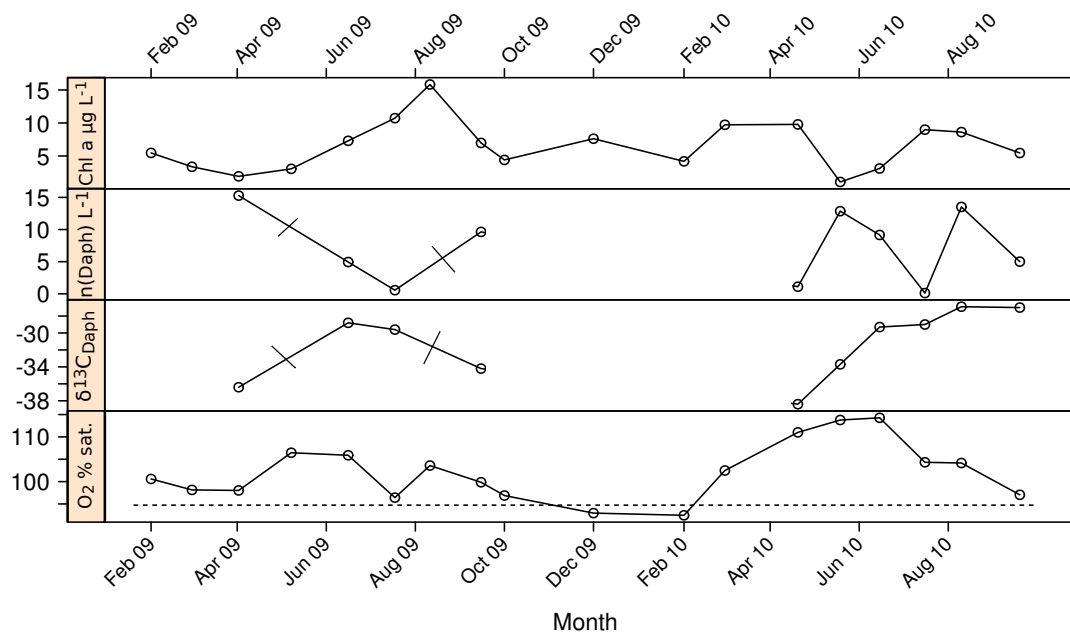


Figure 5.14: Malham Tarn: Productivity, indicated by abundance of *Daphnia* and chl *a*, and oxygen saturation, with $\delta^{13}\text{C}_{\text{Daphnia}}$. Slashes in *Daphnia* data draw attention to the absence of data for May and August 2009, owing to rough weather.

ficient number of data points, certain patterns were detected regarding productivity and $\delta^{13}\text{C}_{\text{Daphnia}}$. The two significant predictors of $\delta^{13}\text{C}_{\text{Daphnia}}$, chl *a* and *Daphnia* abundance,

reflect productivity specifically in the pelagic zone, strongly suggesting that productivity in the pelagic zone forms a significant component of in-lake productivity in Cunswick Tarn. However, determination of whether or not CED/CEF operates is complicated owing to the opposite relationship of *Daphnia* abundance and chl *a* to $\delta^{13}\text{C}_{\text{Daphnia}}$. Under CED/CEF, the correlation between productivity and cladoceran $\delta^{13}\text{C}$ should be negative at peak productivity (Herczeg and Fairbanks, 1987). If *Daphnia* abundance is taken to represent productivity more accurately, CED/CEF is indicated in June data of both years. However, if chl *a* is more representative (as is commonly assumed in isotope studies (Gu et al., 1999) as well as for trophic assessment using TP and chl *a* (Lyche Solheim et al., 2008; Penning et al., 2008), the classic pattern of increasing $\delta^{13}\text{C}$ with increasing productivity holds. Increasing top-down control of phytoplankton by cladocerans, for example by the elimination of planktivorous fish, however, is often successful at reducing standing phytoplankton crops in eutrophic lakes (Carvalho, 1994; Moss et al., 1994; Strand and Weisner, 2001). Also, spring clearwater phases are attributed to strong top-down control of phytoplankton by cladocerans (Lampert et al., 1986; Sommer, 1986; Vanni and Temte, 1990), and zooplankton grazing pressure often explains residual variation of chl *a*-TP relationships (Kamarainen et al., 2008). Therefore, in Cunswick Tarn where (1) *Daphnia* of multiple size classes are abundant, (2) there are no fish, and (3) there is a largely negative relationship between chl *a* and *Daphnia* abundance, *Daphnia* abundance may reflect pelagic productivity more accurately. In June both years, relatively high secchi depths, relatively low chl *a* and peaks in *Daphnia* were coincident, suggesting CED/CEF at these times may have taken place.

Oxygen and pH data, indicating overall productivity rather than productivity in the pelagic zone only, are also slightly ambivalent. The high chl *a* value in July 2009, and the high June 2009/2010 *Daphnia* abundance, occurred when water column O_2 and pH were relatively low at the time of sampling, and therefore the underlying mechanism behind the $\delta^{13}\text{C}_{\text{Daphnia}}$ minima/maxima remains largely moot. It is also entirely realistic that both CED/CEF and ^{13}C enrichment operate in Cunswick Tarn at different times during summer. However, the particularly large drop in O_2 saturation in September 2010, coincident with low $\delta^{13}\text{C}_{\text{Daphnia}}$ and a breakdown of chemical stratification, is taken as a clear indication of uptake of respired carbon (Figures 5.2, 5.12).

It is logical to assume that there are temporal lags involved in the exhaustion of ^{12}C in a

water column. This is indicated by the slight delay in the relatively monotonic increase in the $\delta^{13}\text{C}$ of cladocerans in Hawes Water and Malham Tarn through the growing season with respect to oxygen saturation (Figures 5.13, 5.14). Slow increases are also characteristic of other lakes (Quay et al., 1986; Zohary et al., 1994). The relatively large fluctuations in $\delta^{13}\text{C}$, chl *a* and oxygen saturation in Cunswick Tarn compared with the two other lakes suggests large short-term fluctuations dominate the system, overriding lags associated with “slower” systems. Higher-resolution data, in addition to night-time O_2 information and CO_2 measurements, would have been required to better evaluate autotrophy vs heterotrophy, CED/CEF, and depletion of ^{12}C , in Cunswick Tarn.

5.5.4 Patterns of primary productivity and $\delta^{13}\text{C}$ in the less productive lakes

In contrast to Cunswick Tarn, cladoceran $\delta^{13}\text{C}$ in both Hawes Water and Malham Tarn become consistently higher with time, reflecting reduced fractionation by phytoplankton following hypothesis (Figures 5.13, 5.14). Both lakes also displayed a lag in the increase in cladoceran $\delta^{13}\text{C}$ with respect to productivity as indicated by oxygen saturation, which may reflect a relatively slow process of ^{12}C exhaustion. This is supported by data from Hawes Water in 1998 and 1999, showing an increasing pattern of $\delta^{13}\text{C}_{\text{DIC}}$ in the epilimnion from spring through summer, and reciprocally a decrease in the hypolimnion (J. Marshall, unpubl.). This pattern is in agreement with preferential biological uptake of ^{12}C in the macrophyte/phytoplankton zone over time leading to limitation of ^{12}C , and respiration of ^{12}C in the profundal zone. Also in contrast to Cunswick Tarn, there was no significant correlation between either chl *a* or cladoceran (+ copepod) concentrations and $\delta^{13}\text{C}$ in Hawes Water or Malham Tarn. This indicates productivity in the pelagic zone is likely to be subordinate to macrophytic productivity in the lakes.

The lack of a relationship between chl *a* or cladoceran (+ copepod) concentrations and $\delta^{13}\text{C}$ is somewhat surprising in Hawes Water, since it is a relatively deep lake with only marginal bands of macrophytes and the proportion of primary productivity accounted for by phytoplankton would be expected to be quite high, leading to patterns more similar to those observed in Cunswick Tarn. Other than high macrophyte productivity, there are two alternative explanations for the observed pattern in Hawes Water. It is possible that chlorophyll *a* underestimates pelagic productivity - due to high light penetration, phytoplankton produc-

tivity may extend vertically and surface chl *a* represents only a fraction of the biomass. This is highly likely, as metalimnetic plankton maxima have been recorded in the lake previously (J. Marshall, unpubl.), and subsurface oxygen maxima several meters below the water surface were recorded in 2009 and 2010 (Figure 5.3).

While the exact balance between the pelagic and benthic components cannot be assessed, it is likely that both macrophyte and phytoplankton productivity account for decreasing isotopic fractionation by phytoplankton. For example, overwintering charophytes are able to respond rapidly to increases in photoperiod and temperature in spring, consistent with high O₂ saturation and early onset of carbonate precipitation. The significant relationship between alkalinity and $\delta^{13}\text{C}_{\text{Bosmina}}$ suggests a strong link between $\delta^{13}\text{C}$ and carbonate precipitation, and phytoplankton HCO_3^- uptake no doubt contributes to the build-up of high $\delta^{13}\text{C}_{\text{Bosmina}}$. It is also possible that there are differences in $\delta^{13}\text{C}$ of phytoplankton in the pelagic and the littoral zone, and *Bosmina* represent an integrated signal from both habitats due to horizontal migration as indicated by littoral zooplankton sampling.

Malham Tarn is colonised by abundant macrophytes throughout the lake bed and therefore it was expected that limiting CO₂ would be caused by competition between macrophytes and phytoplankton, rather than an abundance of the latter (Boll et al., 2012). Hence, the lack of a correlation between phytoplankton or *Daphnia* abundance and $\delta^{13}\text{C}_{\text{Daphnia}}$ was not surprising. A dominant macrophyte component is further supported by the correlation between carbonate precipitation (measured as DIC removal) and charophyte growth (Coletta, 2004), and the persistence of macrophytes into autumn (*Potamogeton*, *Chara*, *Elodea* growth recorded in November, *Chara* overwintering). Further, $\delta^{13}\text{C}$ values were particularly high in 2010, when alkalinity and pH data give evidence of carbonate precipitation, compared with 2009 when carbonate precipitation was lower. Macrophyte-mediated ¹²C limitation and HCO_3^- uptake in the pelagic zone are therefore highly likely to be the dominant mechanism behind the increasing trend in $\delta^{13}\text{C}_{\text{Daphnia}}$ in Malham Tarn.

5.5.5 Applicability of $\delta^{13}\text{C}_{\text{eph}}$ and $\delta^{13}\text{C}_{\text{carb}}$ for reconstructing eutrophication in Cunswick Tarn

$\delta^{13}\text{C}_{\text{eph}}$ and $\delta^{13}\text{C}_{\text{carb}}$ were compared with historical productivity patterns of Cunswick Tarn as indicated by core phosphorus, carbonate, pigment, cladoceran and macrofossil data (Fig-

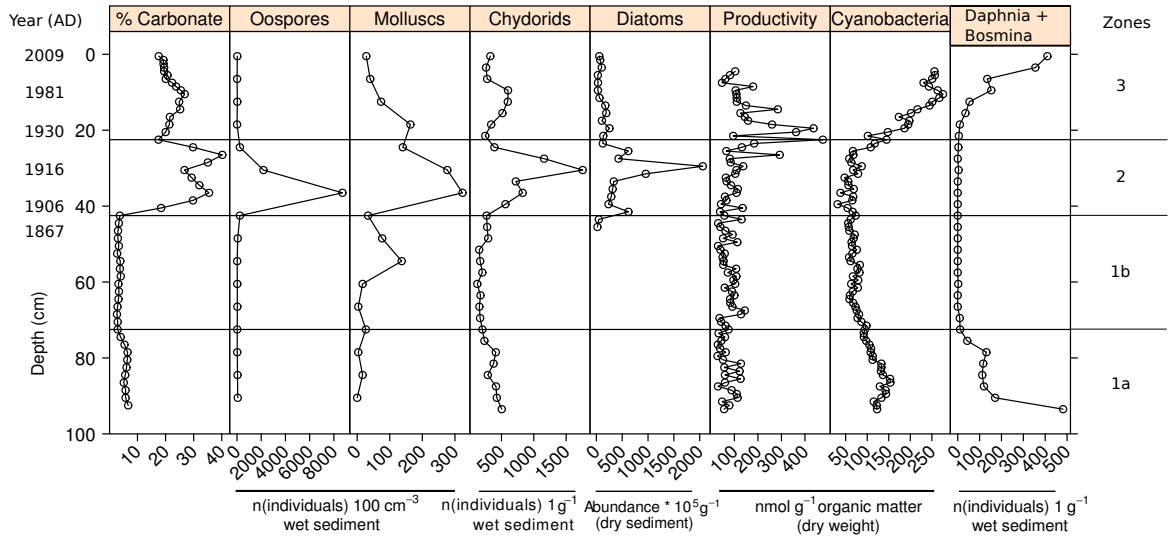


Figure 5.15: Abundances of selected organisms, with “Productivity” referring to total production as given by ubiquitous pigments (see 4).

ure 5.15). Core levels from the base to 72.5 cm (Mediaeval period) encompass a period of relatively high productivity compared with levels further upcore (Chapter 4). By extrapolation from core dating profiles, the productivity pattern of the early period may reflect the Mediaeval Warm Period (AD 880 - 1350 (Dong et al., 2012)), with temperatures comparable to the first half of the 20th century (Ljungqvist et al., 2012). From approximately 70.5 cm to 45.5 - 42.5 cm, productivity is low, with the upper levels corresponding to the end of the Little Ice Age (1850s). Nutrient enrichment and substantial changes in biota have taken place since the late 1800s/early 1900s (upcore from 42.5 cm). Therefore, a high-productivity $\delta^{13}\text{C}_{\text{eph}}/\delta^{13}\text{C}_{\text{carb}}$ signal was expected from the base of the core to approximately 70 cm, reflecting climatic conditions, and upcore of 42.5 cm, reflecting anthropogenic eutrophication. A low-productivity $\delta^{13}\text{C}_{\text{eph}}$ and $\delta^{13}\text{C}_{\text{carb}}$ signal was expected for core levels between these two sections.

Bulk $\delta^{13}\text{C}_{\text{carb}}$

The high variability of bulk $\delta^{13}\text{C}_{\text{carb}}$ of core CUNS2 sediment, and the inconsistency with historical productivity patterns (Figure 5.15), suggests $\delta^{13}\text{C}_{\text{carb}}$ is not amenable to reconstruction of eutrophication. For example, the changes in $\delta^{13}\text{C}_{\text{carb}}$ below 40 cm do not show a relationship with %carbonate and relatively high %carbonate in the uppermost 25 cm, combined with increasing eutrophication, corresponds to relatively stable $\delta^{13}\text{C}_{\text{carb}}$. As for $\delta^{13}\text{C}_{\text{org}}$,

the consistent increase in Suess-corrected $\delta^{13}\text{C}_{\text{carb}}$ since the 1950s may reflect more recent eutrophication, however owing to the large overall variability in values, factors other than eutrophication cannot be ruled out.

Despite the absence of consistent patterns of productivity-driven change in $\delta^{13}\text{C}_{\text{carb}}$, productivity-driven disequilibrium effects in the short term may have occurred. In conditions of low flow, non-equilibrium carbonate precipitation on photosynthesising charophytes can occur (Andrews et al., 2004). Further, large disequilibrium fractionation due to CEF has been observed in ponds with dense beds of *Chara hispida*, resulting in lower-than-expected $\delta^{13}\text{C}_{\text{carb}}$ values (Pentecost et al., 2006). Charophytes were abundant in Cunswick Tarn until at least the 1930s and persisted in lesser abundance until the 1980s, with core levels between 30 and 40 cm (early 1900s) reflecting particularly high charophyte abundance, including *C. hispida*. The effects on $\delta^{13}\text{C}_{\text{carb}}$ of hydrologically closed conditions and variable charophyte productivity through time can not be assessed, and therefore $\delta^{13}\text{C}_{\text{carb}}$ is regarded an ambiguous indicator of lake trophicity in (shallow) high-carbonate lakes with large changes in carbonate deposition over time.

(Leng et al., 2006; Talbot, 1990). *Daphnia* $\delta^{13}\text{C}_{\text{eph}}$

Daphnia $\delta^{13}\text{C}_{\text{eph}}$ also do not show monotonic linear change related to eutrophication, in conflict with the null hypothesis, and variability is apparent through the core (Figure 5.10). However, there are two plausible (and mutually coherent) explanations for the observed pattern, consistent with eutrophication-related trends; macrophyte-mediated ^{12}C competition (Boll et al., 2012), and seasonality of ephippia production (Jankowski and Straile, 2003, 2004). Taking these two factors as the main drivers of $\delta^{13}\text{C}_{\text{eph}}$ in CUNS2 as representative of shallow calcareous lakes, ephippia can be valuable as indicators of early eutrophication-driven change.

Current trends of ephippial production and pelagic/benthic balance in Cunswick Tarn

Daphnia in Cunswick Tarn are currently abundant, and increases early in the season correspond to decreases in chl *a* and a short clear-water period, followed by lesser abundance and increases in *Ceratium*, in agreement with patterns commonly observed in meso- eutrophic lakes (Coveney et al., 1977; Lampert et al., 1986; Sommer, 1986; Vanni and Temte, 1990). As indicated by the correlation between $\delta^{13}\text{C}_{\text{Daphnia}}$ and chl *a* + *Daphnia* abundance, there is a strong relationship between pelagic productivity and ^{12}C consumption. The high abundance of zooplankton and phytoplankton in the lake support the idea of a strong pelagic compo-

nent to whole-lake productivity. Correspondingly, ehippia (and male) production relatively early in the season in June (May) in Cunswick Tarn follows seasonality predicted in lakes with high algal biomass suppressed by cladocerans early in summer (Smyly, 1979; Alekseev and Lampert, 2001). Although autumn (post-September) zooplankton monitoring was not undertaken, it is assumed that ehippia are also produced later in the season when food abundance/quality and temperature decrease (Kleiven et al., 1992; Koch et al., 2009). The abundance of ehippial females in September 2009 is strong evidence for an autumn peak also.

$\delta^{13}\text{C}_{\text{Daphnia}}$ and $\delta^{13}\text{C}_{\text{ehippia}}$ are relatively low throughout the growing season probably owing in part to high respiration, and therefore variation in the balance between spring and autumn ehippial production does not necessarily result in deviation from an “end-of-season” fossil signal. However, if at some point in the past $\delta^{13}\text{C}_{\text{Daphnia}}$ evolution through summer was more similar to what is observed in Hawes Water and Malham Tarn at present, fluctuations in seasonality could have a large effect on the interpretation of fossil $\delta^{13}\text{C}_{\text{eph}}$. Importantly, as demonstrated by Boll et al. (2012), phytoplankton $\delta^{13}\text{C}$ can be particularly high when macrophytes are abundant, and lower when macrophytes are less abundant and/or absent. This may have implications for shallow lakes where eutrophication typically involves a decline of macrophytes, as this would lead to decreasing fossil $\delta^{13}\text{C}_{\text{eph}}$ with eutrophication. In such a scenario, a eutrophication- related shift to increasing abundance of *Daphnia* in spring and therefore early-season ehippia production (Jankowski and Straile, 2003, 2004), with corresponding early-season low $\delta^{13}\text{C}_{\text{eph}}$, would not be in conflict, but rather in synergy, with the macrophyte-related signal. Such a scenario is in strong agreement with the observed fossil $\delta^{13}\text{C}_{\text{eph}}$ values, in contrast with the poor fit of the null hypothesis.

The early warm period, and high productivity in the pelagic zone

In many respects, the core base section reflects conditions similar to Cunswick Tarn at present. Firstly, anoxic habitats were abundant as indicated by core pigment data showing presence of purple sulfur bacteria (core base - 72.5 cm) (Chapter 4). Such conditions are likely to have existed also owing to closed conditions and warmer climate leading to higher thermal stability (Viner, 1985; Foley et al., 2012). Secondly, food was abundant in the pelagic zone as indicated by high numbers of fossil *Daphnia* ehippia and spines, and thirdly, cyanobacteria were also relatively abundant. As such, the similarity between $\delta^{13}\text{C}_{\text{eph}}$ in this core section

and the uppermost core section is in agreement with the alternative hypothesis. It is likely that the $\delta^{13}\text{C}_{\text{eph}}$, as at present, are explained through a prevalence of ephippia production early in the season (Jankowski and Straile, 2003, 2004), a supply of respired $^{12}\text{CO}_2$, and relatively low competition with macrophytes. However, productivity was likely lower in this period than currently, and a more diverse macrophyte and cladoceran community is reflected by palaeolimnological data. These differences suggest it is unwise to assume similar seasonal evolution of $\delta^{13}\text{C}_{\text{eph}}$. In moderately productive conditions, it is likely that $\delta^{13}\text{C}_{\text{Daphnia/eph}}$ become higher with time as observed in Malham Tarn and Hawes Water (as in many other lakes; Zohary et al. (1994); France et al. (1997); Gu et al. (1999)).

Macrophytes, and low productivity in the pelagic zone

The high *Daphnia* $\delta^{13}\text{C}_{\text{eph}}$ from 65.5 to 30.5 cm encompass substantial changes in abundance and composition of biota, and also a change towards higher trophic state, as shown through palaeolimnological analyses (Chapter 4). In agreement with the alternative hypothesis, however, macrofossil analysis (resolution 6 cm) shows high scarcity of *Daphnia* ephippia from core depth of 66.5 cm to 24.5 cm (Figure 5.11), suggesting high $\delta^{13}\text{C}_{\text{eph}}$ may be related to *Daphnia* abundance, and therefore, the proportion of pelagic productivity in the lake. Taphonomy is an unlikely explanation for the scarcity of ephippia, as those found did not show substantial degradation, which also applies to those found at the base of the core. Further, *Daphnia* spine and claw counts show the same pattern (data not shown).

Lowered productivity, possibly due to cooling climate (Little Ice Age), is inferred for core levels above 72.5 cm. Productivity in the pelagic zone largely disappeared as evidenced by the scarcity of *Daphnia* and *Bosmina* remains. However, the abundance of calcite-encrusted charophyte remains and molluscs in the fossil record suggests productivity dominated by benthic communities. The disappearance of the purple sulfur bacterial pigment from the core record indicates the lake may also have become more oxygenated. Such a change could be inferred to indicate diminished contribution of respired $^{12}\text{CO}_2$, thereby explaining the increase in $\delta^{13}\text{C}_{\text{eph}}$ (especially as respiration is more responsive to temperature changes at the low end than photosynthesis; del Giorgio and Peters 1994). However, under the null hypothesis, lowered productivity would be expected to push $\delta^{13}\text{C}$ down owing to less ^{12}C limitation, which is not observed. $\delta^{13}\text{C}_{\text{eph}}$ are also high following the onset of eutrophication at the end of the 19th century (42.5 cm). Therefore, it is unlikely low vs high productivity alone explains the

pattern in $\delta^{13}\text{C}_{\text{eph}}$ through this core section. Interestingly, calcite deposition does also not show a direct relationship with $\delta^{13}\text{C}_{\text{eph}}$. Core carbonate content is particularly high between 42.5 and 24.5, with peaks at 36.5 and 26.5 cm, but the upper peak corresponds to decreasing $\delta^{13}\text{C}_{\text{eph}}$ (Figure 5.10).

The initial stage of eutrophication in the early 1900s is characterised by extremely abundant calcite-depositing charophytes and molluscs, benthic and epiphytic diatoms, and plant-associated cladocerans (Chapter 4). Combined with the scarcity of pelagic cladocerans, this strongly suggests a food web based on benthic (particularly macrophytic) pathways prevailed through the initial stages of eutrophication. Therefore, as before the 1900s, pelagic productivity was suppressed and this commonality between the two trophic phases is most likely to lie behind the high $\delta^{13}\text{C}_{\text{eph}}$ in the mid section compared with the much more pelagically dominated warm period and post-1920s. This is strong evidence in favour of benthic ^{12}C uptake, resulting in suppression of pelagic productivity and high $\delta^{13}\text{C}_{\text{eph}}$ of *Daphnia* (Boll et al., 2012). This is also in agreement with the relatively high $\delta^{13}\text{C}_{\text{eph}}$ and $\delta^{13}\text{C}_{\text{Daphnia}}$ values and macrophyte colonisation in Malham Tarn at present (Figure 5.8).

Finally, it is also possible that the seasonality of ephippia production changed. With less productivity in the plankton, the meso- eutrophic zooplankton abundance pattern involving clear-water phases and algal succession may not be expected to occur to the same degree (Sommer, 1986, although see Carrillo et al. 1995) and ephippial induction could be reduced in comparison to the end of the season. Reciprocally, increased food availability has been linked with lesser end-of-season ephippial production and overwintering, shifting the balance in favour of an early-season ephippia signal (Smyly, 1979; Jankowski and Straile, 2003, 2004). Changes in seasonality are not in disagreement with the macrophyte hypothesis, as both late peaks in ephippia production and high macrophyte biomass are indicators of relatively unimpacted systems.

Macrophytes, and increasing productivity in the pelagic zone

Following the decrease of $\delta^{13}\text{C}_{\text{eph}}$ at 30.5 cm (1916), values remain relatively invariable to the core top (2009) despite high calcite deposition, indicating reduced use of HCO_3^- owing to a source of ^{12}C (as currently in the lake), a change in seasonality back towards spring ephippia production, and/or increases in pelagic productivity. Through the 1920s, the macro-

phyte and cladoceran communities changed, with a shift from a predominance of charophytes to angiosperms, and there was an increase in pelagic cladocerans (Chapter 4). Slightly less dense macrophyte biomass during this period is indicated by an increase in benthic diatom species relative to epiphytic species. Cunswick Tarn was, however, still predominantly a benthic system, and the abrupt change in $\delta^{13}\text{C}_{\text{eph}}$ is slightly unexpected and higher-resolution data would be desirable. Clear indication of eutrophic conditions, substantial loss of charophyte communities, and a shift to increasing proportions of pelagic productivity (substantial increase in *Daphnia* remains), are only indicated by other palaeolimnological data closer to the 1930s (above a core depth of 24 cm). Further, this change is gradual, in contrast to the steplike change in $\delta^{13}\text{C}_{\text{eph}}$.

It is unlikely that respired ^{12}C was substantial before the 1920s, as there is no evidence of anoxia such as presence of purple sulfur bacteria or a decline in molluscs (in contrast to after the 1930s). Therefore, the change is more likely to be a combination of a change in seasonality of ephippia production and decreased competition for carbon from macrophytes. Either or both factors, or some unknown, may exhibit a relatively low threshold for change thereby providing an “early warning” eutrophication signal. Unfortunately, analysis of more core levels was beyond the scope of this study. Seasonal data of similar lakes would be valuable to confirm that $\delta^{13}\text{C}$ changes recorded by cladocerans indeed can indicate changes in the balance between macrophyte and phytoplankton production.

Through most of the core, $\delta^{13}\text{C}_{\text{eph}}$ of *Simocephalus*, a plant-associated cladoceran, are higher than $\delta^{13}\text{C}_{\text{eph}}$ of *Daphnia*. The reason for including *Simocephalus* in this study was mainly to establish whether the two filter-feeding Daphniidae could be amalgamated, rather than to investigate causation behind any differences revealed by the data. As such, this study shows that specificity in isotope studies is required in order to yield data on past environments, as opposed to noise resulting from conflicting signals. The difference in $\delta^{13}\text{C}$ between the taxa may result from reduced fractionation under boundary conditions in the macrophyte zone (Hecky and Hesslein, 1995), where *Simocephalus* feeds. Alternatively, the two taxa have consistent and divergent food source preference, as indicated for copepods and *Daphnia* (Matthews and Mazumder, 2003; Lee et al., 2011). subscripteph of *Daphnia* become higher (65.5 - 30.5 cm), exceeding $\delta^{13}\text{C}_{\text{eph}}$ values of *Simocephalus* at 42.5 cm.

5.5.6 Conclusions

1. The first hypothesis of the study was partially met. A consistent positive change in cladoceran $\delta^{13}\text{C}$ over the summer season in the mesotrophic lakes (HW, MT) was observed, reflecting decreasing supplies of ^{12}C due to biological uptake and also increased HCO_3^- use. However, change was not consistent in Cunswick Tarn as initially put forward, and cladoceran $\delta^{13}\text{C}$ were low overall. Relatively high values corresponded to short-term phytoplankton blooms under reduced predation pressure, and low values corresponded to periods of high predation pressure and seemingly high respiration, thereby leading to ambivalent indications of CED/CEF and/or reduced fractionation. These data suggest that in eutrophic lakes with low throughflow and high contributions of respired carbon in the hypolimnion, eutrophication signals of increasing anoxia and carbon limitation conflict, and changes in resulting $\delta^{13}\text{C}_{\text{org}}$ in the sediment may be limited or ambivalent. The low values encountered in Cunswick Tarn are in agreement with the eutrophication scenario of Hollander and Smith (2001) where at intermediate TP concentrations microbial processes dominate the $\delta^{13}\text{C}$ signal.

2. The null hypothesis of increasing $\delta^{13}\text{C}_{\text{clado}}$ with increasing lake trophity did not hold, again in part due to microbially mediated ^{12}C in Cunswick Tarn. Respired carbon from the hypoxic/anoxic hypolimnion may also have contributed to the relatively low $\delta^{13}\text{C}$ of *Bosmina* in Hawes Water. In support of the findings of Boll et al. (2012), Malham Tarn, with the highest standing biomass of macrophytes, had higher $\delta^{13}\text{C}_{\text{clado}}$ values than both Cunswick Tarn and Hawes Water, even when accounting for the maximal difference found between *Bosmina* and *Daphnia*.

3. $\delta^{13}\text{C}$ of fossil *Daphnia* ephippia did not follow the null hypothesis of increasing $\delta^{13}\text{C}$ with eutrophication. However, the data support the alternative hypothesis regarding eutrophication-driven changes in ephippia production and macrophyte biomass whereby increases reflect increasing macrophyte biomass and an end-of-season ephippial pulse, and decreases reflect decreasing macrophyte biomass and an increasingly dominant early-season ephippial pulse.

$\delta^{13}\text{C}$ of fossil ephippia revealed change that was not apparent in bulk sediment isotope analyses, thereby opening up new avenues of research. More studies assessing the relationship between macrophyte abundance, and seasonal patterns of $\delta^{13}\text{C}$ and ephippial production in cladocerans are required to assess the reproducibility and reliability of the fossil signal indi-

cated in this study. Also, owing to the variability in $\delta^{13}\text{C}_{\text{eph}}$, palaeolimnological data should ideally consist of several replicates and/or individual data points consisting of a large number of ephippia. More comprehensive studies than the one undertaken here, comparing $\delta^{13}\text{C}_{\text{eph}}$ of pelagic and plant-associated cladocerans, could also give valuable insight into the differences between taxa and the relationship of these differences with environmental conditions. Finally, high-resolution data sets would allow more statistically meaningful evaluation of factors contributing to cladoceran $\delta^{13}\text{C}$.

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6 Summary, conclusions, and management implications

6.1 Introduction

The overarching aims of this PhD project were to elucidate the nature, timing and magnitude of eutrophication-driven changes in biological and chemico-physical processes of marl lakes on a centennial scale, and thereby establish their sensitivity to enrichment. Comprehensive contemporary monitoring of the sites was also undertaken in order to combine the past and the present, one informing the other. The aim was subsequently also to inform the assessment of the ecological status and reference conditions of marl lakes as required by the EU Water Framework Directive (WFD), for lake management and conservation purposes.

The assessment of ecological status using WFD metrics was the focus of chapter 2, undertaken for all three study sites by comparing classifications based on contemporary data with those based on historical and palaeolimnological records. The wealth of data allowed the characterisation of lake-specific ecological change through time, and control for seasonal variability.

The eutrophication-driven changes were investigated in more detail by way of multi-proxy palaeolimnological studies of Hawes Water and Cunswick Tarn in chapters 3 and 4, respectively, which showed changes in all groups under study (macrophytes, microphytes, molluscs, trichopterans and cladocerans). An investigation into the applicability of stable isotope data of fossil *Daphnia* ephippia in eutrophication studies indicated that the method is a promising new palaeolimnological tool although further development is needed (chapter 5).

Key findings with respect to the original research questions and hypotheses are summarised below, along with suggestions for further research.

6.2 Current and reference ecological status of the study sites

As predicted, macrophyte species richness declined with increasing nutrient enrichment within and across all three study sites, and the temporal changes in species composition were in good agreement with those expected across a gradient of enrichment (Willby et al., 2010). Palaeolimnological data for both Hawes Water and Cunswick Tarn revealed increases in production in the pelagic zone with time. Further, macrophyte dieback was found to occur relatively early in Cunswick Tarn, the most impacted site, compared with Hawes Water and Malham Tarn where elodeids persist late into the year, and charophytes overwinter.

Two major patterns were revealed. Firstly, in agreement with other studies, charophytes were shown to be sensitive to eutrophication, but importantly, their sensitivity to light climate became especially apparent. Charophyte losses, both in terms of spatial representation and species richness, have occurred in Cunswick Tarn and Hawes Water, both of which are steeply shelving kettlehole lakes and therefore very responsive to changes in light penetration in terms of macrophyte depth zonation. This is exemplified by the decadal-scale reduction in the colonisation depth of *Chara rudis*, finally leading to its extirpation from Hawes Water, and the complete absence of charophytes from Cunswick Tarn at present despite flourishing communities in the first half of the 20th century. In Malham Tarn, where there are no steep slopes and maximum water depth is only around 4 m, charophyte communities have remained largely the same as those observed in the early 1900s.

The other pattern involves the loss of diversity within the Potamogetonaceae, which has occurred in all lakes and has affected Malham Tarn the most, with seven historically recorded *Potamogeton* now absent. The species lost in all lakes include those typically associated with nutrient-poor lakes, and those remaining, with nutrient-rich lakes. It is also noteworthy that *Littorella uniflora*, a typical shoreline species, has been historically recorded in both Hawes Water and Malham Tarn, revealing losses also in the shallow water habitat, likely through siltation.

Palaeolimnological and historical data were in agreement with the depiction of marl lakes in reference status (Willby, 2005; Willby et al., 2010). A diverse community of particularly Characeae and Potamogetonaceae was indicated for all study sites, including the presence of *L. uniflora* in Malham Tarn and Hawes Water. Pre-enrichment conditions in Cunswick Tarn (pre-1900s) were characterised by high water transparency, low overall productivity, and charophytes in all depth zones as revealed by analysis of two cores from the lake. Marl-encrusted *Chara aspera* was abundant in the littoral zone, and deeper zones contained a mixture of *Nitella* sp., *Chara hispida* agg., and *Chara contraria* agg. The relatively low number of molluscs and macrophyte-associated chydorids indicates relatively low plant cover, in agreement with relatively low plant cover also indicated for Malham Tarn in the 1930s and 1960s compared with the present.

When applied to the study sites, the performance of individual WFD metrics varied by lake type. While indices based on productivity in the pelagic zone were not deemed appropriate

for characterising Malham Tarn, very shallow by WFD type, the opposite was true for Hawes Water and Cunswick Tarn, both shallow by WFD type. The assessment of Malham Tarn is in support of the total phosphorus (TP) boundaries for marl lakes suggested by Free et al. (2007). In general, the simple indices (TP, chlorophyll *a* and colonisation depth), and the raw values of species richness and the Lake Macrophyte Nutrient Index LMNI, were more robust than Ecological Quality Ratios (EQRs), suggesting EQRs should be further developed before being applied. In summary, the study suggests that Cunswick Tarn, Malham Tarn and Hawes Water are currently in Bad, Moderate, and Moderate status, respectively.

6.3 Marl formation over time and increasing eutrophication

It was predicted that high sediment carbonate content would be associated with an abundance of *Chara*, and declining *Chara* beds in turn with a decrease in carbonate content. This association is supported by the Hawes Water and Cunswick Tarn core data (Chapters 3 and 4). Core carbonate content was high throughout the HAWE3 profile, as were *Chara* stem remains. In Cunswick Tarn, maximal carbonate content was associated with high oospore numbers as well as stem remains. Carbonate content in Cunswick Tarn declined upcore where both historical and palaeolimnological data indicated decreases in *Chara* cover. Further, a marl-organic transition was evident in several cores collected over the study period, in support of the hypothesis of increasingly organic sediment composition with eutrophication. Data from cores collected from other sites also show the same pattern (e.g. Llyn yr Wyth Eidion, Wales, shown with a core from Hawes Water in Figure 6.1).

Unfortunately, historical marl formation patterns could only be investigated for Hawes Water and Cunswick Tarn, as sediment cores collected from Malham Tarn did not contain recent sediment amenable to radiometric dating. A successfully dated core was retrieved from Malham Tarn in January 2011, and, while time constraints prevented analysis within this PhD project, there is potential to examine this core in the future to establish whether patterns seen in Cunswick Tarn and Hawes Water are also evident here (in fact pigments have already been analysed in collaboration with S. McGowan, though data have not been processed).

The analysis of *Chara* fossil remains gave rise to new questions. In both Hawes Water and Cunswick Tarn, upcore decreases in oospore numbers were associated with increases in sediment pigment concentrations, suggesting that charophyte fertility may be very sensitive to



a Llyn yr Wyth Eidion



b Hawes Water.

Figure 6.1: A very apparent transition from marly to organic sediment in a core from Llyn yr Wyth Eidion, Wales, and a deep water core from Hawes Water.

prevailing light conditions. In Cunswick Tarn, oospore abundance followed a similar pattern to stem remain abundance, however this was not the case in Hawes Water where stem remains were extremely abundant to the very top of the core in agreement with historical records of *Chara* on site. This shows that oospore abundance is not necessarily correlated with *Chara* abundance, which should be taken into consideration when interpreting fossil records. In marl lakes with well preserved stem remains, however, this pattern does not cause problems, but rather offers new avenues of research into the relationship between charophyte abundance, fertility, light climate, and eutrophication.

Although marl lakes are in part defined by the precipitation of calcite, a detailed study of calcite precipitation was beyond the scope of this study. Therefore, the relationship between *Chara* abundance, and the summertime inverse pattern of alkalinity and pH, remains poorly understood. The seemingly clear pattern in lakes of good status, and noisy or absent pattern in more impacted lakes, needs to be explored in future work.

6.4 Nature, timing and magnitude of change in marl lakes

The use of multiple palaeolimnological proxies proved invaluable for interpreting ecological change in Hawes Water and Cunswick Tarn, and allowed detailed investigation of the timing of responses across biological groups in Cunswick Tarn with the support of historical records. In agreement with the initial hypothesis, abundant charophyte remains characterised core levels corresponding to times of relatively low impact, and charophyte remains were among the first indicators of change with the onset of eutrophication.

Patterns of change

Early responses to eutrophication were evident in both Cunswick Tarn (early 1900s) and Hawes Water (early 1800s). In Hawes Water, the early change involved a marked decrease in oospores, which coincided with increases in pigment concentrations thereby suggesting possible threshold responses of charophyte fertility to light conditions. This step-like change notwithstanding, eutrophication-driven change was initially relatively gradual. Pigment concentrations progressively increased between the early 1800s and the mid-1900s, and historical records reveal diverse communities of the Characeae and Potamogetonaceae throughout the first half of the 1900s. Early change in Cunswick Tarn was more abrupt, occurring within a decade in the early 1900s involving synchronous increases in marl deposition, macrophytes, molluscs and plant-associated cladocerans. The charophyte community was diverse and oospore production was high (up to 20-fold from pre-enrichment levels).

In both lakes, there was an absence of pelagic productivity during the early stages of eutrophication, with low numbers of pelagic cladocerans in the macrofossil record of Cunswick Tarn, and none in that of Hawes Water. In both lakes, multivariate analyses revealed that stratigraphic changes were well defined, and clusters were fairly similar between pigment and macrofossil data of both sites, with synchronicity also demonstrated in Cunswick Tarn for marl deposition and cladoceran assemblages. These data suggest coherent ecosystem-level change in both lakes.

Qualitatively, the most striking change in Hawes Water concerned the microphytes, with a succession first from nitrogen-fixing cyanobacteria to chlorophytes and then to non-nitrogen fixing cyanobacteria, all within the 1950s and the 1960s. The chlorophyte-cyanobacterial turnover corresponded with a marked increase in the remains of Nymphaeaceae and cladocer-

ans implying simultaneous taxonomic and abundance changes among microphytes (epiphyton and phytoplankton) matched with increases in production in the pelagic zone and an increase in floating-leaved macrophytes. These data therefore reveal a relatively rapid ecosystem-level response involving a shift from predominantly benthic to more pelagic production (Vadeboncoeur et al., 2003).

Also in Cunswick Tarn, the most striking change concerned benthic-pelagic shifts. A step change in the operation of benthic buffering of enrichment is suggested by the decrease in core carbonate content and increase in pigment concentrations including those of cyanobacteria coincident with a substantial decrease in charophyte remains. Again, substantial changes are apparent in plant, mollusc and cladoceran communities, although there is less synchronicity across groups. The relatively early loss of the benthic pathway (Vadeboncoeur et al., 2003) demonstrates the sensitivity of marl lakes to enrichment. However, despite the step change, a rapid degradation to turbidity and complete phytoplankton dominance did not occur. Rather, this last phase of increasing pelagic productivity is ongoing and has therefore developed over approximately the last 80 years.

Interestingly, the most recent historical data (1980s - 2010) of both Hawes Water and Cunswick Tarn suggest that ecological responses to eutrophication may be accelerating. In both lakes, severe declines in charophytes took place over the 1980s, in Cunswick Tarn involving the complete extirpation of charophytes, and in Hawes Water, the extirpation of *Chara rudis* through reductions in colonisation depth. In the uppermost levels of both cores, there are substantial increases in *Daphnia* remains. In Hawes Water, this associates with a substantial increase in Nymphaeaceae remains and a decline in the previously abundant cyanobacterial pigments. Phytoplankton production recorded in the water column as chlorophyll *a* has approximately doubled over the same time period, suggesting a further ecological upset may be underway. In Cunswick Tarn, the *Daphnia* increase associates with a dramatic loss in macrophyte species diversity and recent changes in the diatom assemblage.

A note on water levels

Water level changes have occurred in both Hawes Water (recorded) and Cunswick Tarn (inferred) due to land drainage, and because land drainage was common practice when improving land for agriculture, eutrophication and water level changes can be simultaneous and therefore

the effects may be difficult to disentangle. For example, both a decrease in water depth and eutrophication might be expected to lead to increasing oospore production and macrophyte abundance. Throughout the thesis, this matter was only dealt with cursorily, essentially because the eutrophication signal was so strong. In Cunswick Tarn, biotic community and carbonate deposition patterns were similar in both sediment cores from the lake despite a 1.5 m difference in coring depth, and a littoral signal in the shallow-water core was apparent prior to the water level change. Further, increase in core phosphorus content and pigments argued in favour of eutrophication, especially as decreases in water level allow more light to reach the sediment, resulting in photodegradation and lower pigment concentrations.

In Hawes Water, the matter is slightly more debatable because no isotope data were available to pinpoint the change in water level to a specific sediment core level. If the period of high influx of terrestrial matter corresponded to lower water levels than during subsequent periods of lower terrestrial influx, increasing water depth may explain the decrease in oospores, and increasing pigment concentrations, over time. However, this was assumed not to be the case, as the land drains are still present in the catchment, and similar numbers of oospores were not recorded following historical records of redigging in the 1960s. It would, however, be reassuring if similar oospore-pigment-eutrophication patterns as those observed in Hawes Water and Cunswick Tarn were confirmed in lakes where water level changes are absent.

6.5 The ecological response of marl lakes to enrichment: summary

The enrichment gradient of the three study sites is relatively small compared with for example the numerous highly enriched lakes in the Netherlands and Denmark, and therefore the full procession of eutrophication could not be explored. Indeed, none of the study sites have lost all macrophytes and become turbid. Shortcomings notwithstanding, several key processes were identified, summarised by the following sequence along a gradient of enrichment:

Reference status in marl lakes is characterised by high water transparency, and a diverse community of Characeae and Potamogetonaceae with a predominance of benthic production, and suppressed planktonic production.

Initial responses to eutrophication involve increases in macrophyte biomass and associated primary consumers, increased marl deposition, yet possibly decreased charophyte fertility. Overall, high water transparency and low production in the plankton persists.

The functioning of marl lakes becomes compromised with continuing enrichment. Reductions in macrophyte colonisation depth and diversity serve as early warnings, followed by increases in pelagic production and increasing rates of change, possibly also upsetting the marl precipitation pattern in the benthos. Charophytes can disappear completely, leading to the loss of the most characteristic feature of marl lakes and therefore to questionable classification as a marl lake. Charophytes and the Potamogetonaceae, and calcareous geology, are widely distributed across the globe (Stauffer, 1985; Ishaq and Kaul, 1988; Stabel, 1986; Krienitz et al., 1996; Krolikowska, 1997; Wieland et al., 2001; Samu et al., 2010; Ludovisi and Gaino, 2010; Korponai et al., 2011), and calcareous lakes in Europe are classified as a separate lake type (EU-HAB 1992; EC-DG ENV 2007). The loss of charophytes and shallow marl lake functioning with eutrophication as found in this study, is therefore highly likely to take place globally and should be addressed given the high macrophyte species diversity in unimpacted marl lakes.

The evidence for acceleration in eutrophication over the last three decades in Hawes Water and Cunswick Tarn may indicate that marl lakes can show a specific pattern of response (see e.g. ecological patterns discussed in Andersen et al., 2009), and it would be extremely valuable to continue monitoring these sites to establish whether the predicted trajectory of continuing and accelerating colonisation depth and species losses takes place. Such data would aid in concluding the story of enrichment shown through this study. Future research should include larger trophic gradients to enable more robust conclusions to be drawn regarding the buffering capacity of marl lakes.

6.6 Management implications

While eutrophication research is valuable in its own right as a contribution to freshwater science, the knowledge gained can and should be communicated and applied to the real world. The experience gained through combining palaeolimnology with contemporary monitoring is of great benefit to the management and conservation of water bodies. For example, the degradation process identified in Cunswick Tarn and Hawes Water highlights the need to conserve British marl lakes. At present, numbering around 100, they constitute around 1% of all British lakes. However, it is possible that there are many more, overlooked owing to an altered non-marl appearance following eutrophication.

All three study sites have changed substantially over the last few centuries, and some of the key changes occurred relatively early (pre-1800s, early 1800s). These data show that shallow marl lakes are highly sensitive to eutrophication, and support the bleak projections of very restricted future occurrences of healthy marl lakes with *Chara* marl formation (Pentecost, 2009). While the number of marl lakes in Britain is low, they are comparatively common in Ireland where their sensitivity to eutrophication has been highlighted (Free et al., 2007). It is advisable also in the UK to set supporting TP boundaries of H/G $7 \mu\text{g L}^{-1}$ and G/M $10 \mu\text{g L}^{-1}$ for marl lakes as suggested by Free et al. (2007). These are lower than the suggested values for both shallow and very shallow marl lakes for the UK (Carvalho et al., 2006), however, in view of the ecological changes in the three study sites, are more relevant. Due to the lesser effects of changes in light penetration in shallower lakes, the retention of three depth classes for marl lakes is advisable.

High maximal colonisation depths are key to maintaining flourishing charophyte communities in shallow (and very shallow) marl lakes with macrophyte depth zonation. Therefore, colonisation depth should be included in WFD assessment of marl lakes. Ideally the maximal colonisation depth of charophytes and angiosperms would be recorded separately in order to identify possible shifts in the community in response to deteriorating light conditions. Without surveying colonisation depths in more marl lakes, setting specific boundaries is difficult, however based on colonisation depths in for example Loch Borralie and those in Hawes Water in the past, those for charophytes suggested by Carvalho et al. (2006) seem appropriate.

The changes in marl lake macrophyte community composition along an ecological gradient as delineated by Willby (2005) (see chapter 2) are largely mirrored in the three study sites, and should therefore be consulted when assessing marl lakes. Therefore, marl lakes should have a diverse *Potamogeton* and charophyte community, and relatively low abundances of species indicating eutrophic conditions (Willby, 2005). Within this general framework, site-specific compositions may be expected, as for example in the exposed Malham Tarn, at 375 m a.s.l., there are neither well-developed reedswamps nor lily beds, compared with Hawes Water, where both are abundant as is also *Potamogeton coloratus*, a species associated with slow-flowing water and ditches.

It is regrettable that more protection has not been awarded to Cunswick Tarn, Hawes Water, and Malham Tarn, especially as they are all Sites of Special Scientific Interest, and the

latter two also lie within nationally recognised nature reserves. In Malham Tarn, where the inflow region in particular is degraded, the recent nutrient budget work is commendable. A nutrient budget should also be developed for Hawes Water, as the most significant nutrient sources have yet to be identified. In Cunswick Tarn, there are directly observable nutrient sources such as the drain pipe from improved pasture, grain deposited into the water, and the artificially large waterfowl population. The detrimental effect of nutrient loading on all three lakes should be acted upon, as recovery is often a long-term process and the WFD deadline of 2015 for achieving Good surface water ecological status is approaching.

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